EFFECT OF *CHANNA STRIATUS* ON INFLAMMATORY MARKERS AS AN ADJUNCT TREATMENT IN ALLERGIC RHINITIS – A RANDOMIZED DOUBLE-BLINDED STUDY

by

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In the Name of Allah, the Most Beneficient, the Most Merciful. They (angels) said: “Glory be to You, we have no knowledge except what You have taught us. Verify, it is You, the All Knower, the All Wise (2:32)

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LIST OF ABBREVIATIONS

ALA	=	Alpha linolenic acid
ALT	=	Alanine transferase
ALT	=	Alanine phosphatase
APCs	=	Antigen presenting cells
AR	=	Allergic rhinitis
AST	=	Aspartate transaminase
DHA	=	Docosahexaenoic acid
ECP	=	Eosinophil cationic protein
EDN	=	Eosinophil-derived neurotoxin
EPA	=	Eicosapentaenoic acid
FBC	=	Full blood count
GMCSF	=	Granulocyte macrophage colony-stimulating factor
ICAM	=	Intercellular adhesion molecule
IgE	=	Immunoglobulin E
IL	=	Interleukin
INF	=	Interferon
LFT	=	Liver Function Test
LTB_4	=	Leukotriene B_4
MBP	=	Major basic protein
MCP	=	Monocyte chemotactic protein

MIP	=	Macrophage Inflammatory Protein
PG	=	Prostaglandin
PMBC	=	Peripheral blood mononuclear cells
PUFAs	=	Polyunsaturated fatty acids
RANTES	=	Regulated upon activation normal T expressed and secreted
RFT	=	Renal Function Test
TGF	=	Transforming growth factor
Th1	=	T-helper 1
Th2	=	T-helper 2
TNF	=	Tumour Necrosis Factor
TNSS	=	Total Nasal Symptoms Score
VCAM-1	=	Vascular cell adhesion molecule -1

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ABSTRAK

Pengenalan: *Channa striatus* (ikan haruan) telah terbukti dalam kajian-kajian terdahulu dapat mengurangkan keradangan dan kesakitan. Kajian ini bertujuan untuk menyelidik kesan anti radangnya terhadap proses radang dalam alahan hidung.

Objektif: Untuk mengkaji kesan ekstrak ikan haruan ke atas tahap kandungan petunjuk keradangan alahan iaitu eosinofil dan interleukin-4 di dalam serum pesakit serta Markah Keseluruhan Simptom-simptom Hidung mereka.

Rekabentuk kajian: Sebuah kajian rawak uji buta ganda dua, plasebo-kawalan, perbandingan klinikal dalam kumpulan selari selama enam minggu. Seramai empat puluh enam pesakit telah dibahagi secara rawak untuk menerima rawatan selama enam minggu menggunakan ekstrak ikan haruan ataupun plasebo. Pesakit diperiksa secara klinikal pada minggu permulaan, ke dua dan ke enam. Darah diambil pada permulaan dan akhir rawatan bagi memeriksa tahap eosinophil dan interleukin-4 di dalam serum mereka.

Keputusan: Kajian menunjukkan pengurangan dalam kadar yang signifikan bagi tahap eosinofil di dalam serum di kalangan kumpulan yang menerima rawatan ekstrak ikan haruan ($p<0.05$). Namun selepas analisa perbandingan dibuat di antara kedua-dua kumpulan rawatan, perbezaan puratanya menunjukkan hasil yang tidak signifikan secara

statistik. Keputusan bagi tahap interleukin-4 di dalam serum juga menunjukkan hasil yang tidak signifikan secara statistik bagi kedua-dua kumpulan. Perbezaan dalam pengurangan Markah Keseluruhan Simptom-Simptom Hidung di dalam kedua-dua kumpulan hanya signifikan bagi kumpulan pesakit yang mengalami simptom yang sederhana teruk dan teruk tetapi tidak bagi ringan.

Kesimpulan: Walaupun ikan haruan hanya menunjukkan kesan modulasi imun yang sedikit di dalam ujian makmal, akan tetapi fungsinya sebagai rawatan tambahan dapat mengurangkan simptom alahan hidung secara signifikan bagi penghidap yang bersimptom sederhana teruk dan teruk.

ABSTRACT

EFFECT OF *CHANNA STRIATUS* ON INFLAMMATORY MARKERS AS AN ADJUNCT TREATMENT IN ALLERGIC RHINITIS – A RANDOMIZED DOUBLE-BLINDED STUDY

Introduction: *Channa striatus* (snakehead fish) have been shown to have anti-inflammatory and anti-nociceptive properties. This study aimed to discover the effect of its anti-inflammatory property on the inflammatory process in allergic rhinitis.

Objective(s): To determine the effect of *Channa striatus* extract on patients' allergic rhinitis inflammatory markers, namely eosinophil and interleukin-4 level and their Total Nasal Symptoms Score.

Methodology: A 6 weeks, randomized double-blinded placebo-controlled trial, parallel group comparative clinical study. Forty six allergic rhinitis patients were randomized to receive *Channa striatus* extract capsules or placebos for 6 weeks. Patients were assessed clinically at weeks 0, 2 and 6. Blood for serum eosinophil and interleukin-4 were taken at initial and final visit and the results were compared.

Results: The result showed significant decrement of serum eosinophil level in *Channa striatus* group ($p<0.05$) however the comparative analysis of the mean difference between two groups was not statistically significant. The result for interleukin-4 level showed no statistically significant changes in either group. The Total Nasal Symptoms Score reduction in both group are significant and comparative analysis of the mean difference between two groups was statistically significant for those with moderate and severe symptoms.

Conclusion: Even though *Channa striatus* showed nonsignificant additional immunomodulation effects in laboratory analysis, however its role as an adjunct treatment in allergic rhinitis did show significant reduction in TNSS for patients with moderate and severe symptoms.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Allergic rhinitis (AR) is a clinical condition where hypersensitivity of nasal mucosa occur on reaction toward foreign substances. The nasal mucosa inflammatory changes are mediated through IgE antibodies. It is a global problem and became a major concern in industrialized world due to its impact on patients' quality of life. Allergic rhinitis sufferers might have poor productivity, lack of sleep and emotional disturbances. For children that suffer from allergic rhinitis, they might miss their school, lack of concentration during study or causing humiliation among their playmates. To control the symptoms is also costly let alone to cure it.

It is estimated around half billion of the world population might suffer from allergic rhinitis (Bousquet *et al*, 2008). In Asia Pacific, a cross-national study on allergies showed that 37% of Malaysian population was having allergic rhinitis (Katelaris *et al*, 2011). In the state of Kelantan, its prevalence among shool children age 12-14 years old was 38.2% (Quah *et al*, 1997). The most common allergen is aeroallergen, especially house dust mites. Around 73% percent of the studied population has sensitization toward two species of dust mites, *Dermatophagoides pteronyssinus* and *Blomia tropicalis* (Yeoh *et al*, 2003).

Clinically, allergic rhinitis consists of four cardinal nasal symptoms, which are nasal obstruction, rhinorrhea, repeated sneezing and itchiness. The presence of at least two of

these symptoms for more than one hour on most days is suggestive of allergic rhinitis (Bousquet *et al,* 2008). According to Allergic Rhinitis and its Impact on Asthma (ARIA) Guidelines, allergic rhinitis can be divided and classified into mild intermittent, moderate-severe intermittent, mild persistent and moderate-severe persistent (Bousquet *et al,* 2012).

The best preventive control for allergic rhinitis is avoidance of allergen. The mainstay treatment for allergic rhinitis is medication. The treatment is given according to the severity of the symptoms. They consist of intranasal corticosteroids, oral anti-histamines, nasal decongestant and mast cell stabilizers. Immunotherapy should be considered for those with symptoms that are not well controlled with medication.

For some patients, due to unsatisfactory response of their illness to these conventional medications, they tried to seek alternative treatment. The use of natural products has become an alternative treatment option for patients in the Western World. Clinicians have raised their concerns over the efficacy and possible side effects. Various herbs have been used in trials to treat patient with allergic rhinitis, such as *Cinnamomum zeylanicum, Malphighia glabra, Bidens pilosa, Nigella sativa, Capsicum oleous* and *Shi Bi Lin* (Ghazi-Moghadam *et al*, 2012).

In Malaysia, *Channa striatus* (snakehead fish) or locally known as haruan has been used traditionally in post-partum mother and post surgery. It is believed to make the recovery

faster and promote wound healing. Various studies were done locally and proved that this natural remedy indeed has the potentials to promote wound healing, anti-inflammatory and anti-nociceptive properties (Mat Jais, 2007). In our study, we tried to explore on the benefit and the anti-inflammatory property of the fish in treating allergic rhinitis.

1.2 Pathophysiology of Allergic Rhinitis

Allergic rhinitis is an inflammatory reaction comprises four phases – sensitization, early phase reaction, late phase reaction and systemic activation.

1.2.1 Sensitization

During initial phase of allergic rhinitis, antigens such as house dust mite, pollen and animal dander are deposited in the nasal mucosa. In the mucosa, these antigens are engulfed by antigen presenting cells (APCs) mainly Langerhans cells. Inside the APCs, polypeptide products of antigen are partially degraded into fragments. These fragments are then presented/expressed on the surface of APCs together with major histocompatibility complex to be recognized by naïve T-cells ($CD4^+$). These T-cells then is activated, proliferated and differentiated into more specific Th2 cells. Once activated, Th2 cells secrete various cytokines such as IL-4, IL-5, IL-9 and IL-13. IL-4 and IL-13 (but with lower magnitude) are responsible for differentiation and growth of Th2 cells and B-cells activation. They help the B-cells to develop into plasma cells and induce their antibody class switching to IgE (Flint *et al*, 2010). Furthermore, IL-4 mediates the expression of

vascular cell adhesion molecule-1 (VCAM-1), promotes transmigration of eosinophil across endothelium and increase mucus secretion and more cytokine release from Th2 cells (Steinke & Borish, 2001). Meanwhile, the secreted IL-5 is responsible for eosinophil proliferation, its chemotaxis and survival and the IL-9 induces mucus production and mast cells proliferation (Flint *et al*, 2010). The produced IgE then is bound to the cells that posses a specific receptor for Fc part of the IgE mainly mast cells. These well-armed mast cells now ready for the next phase of reaction (Fig.1).

1.2.2 Early Phase Reaction

Following exposure to the allergen, allergic inflammatory response takes place in the sensitized individuals. This occurs within minutes after the exposure. Allergen in the airway mucosa cross-links with IgE receptors on mast cells surface. Interaction between the allergen and immunoglobulin E causes subsequent release of mediators such as histamine. The histamine release causes mucus hypersecretion, an increase in vascular permeability and mucosal oedema (Fig.1). This may result in rhinorrhea, nasal congestion, sneezing and itching sensation in patient who suffer from allergic rhinitis (Flint *et al*, 2010).

Beside histamine release, mast cell degranulation also lead to the release of other mediators and cytokines. From its membrane phospholipids, through the arachidonic acid pathways, leukotrienes and PGD_2 are generated. Leukotrienes have their role in causing the increase vascular permeability and oedema of the nasal mucosa, eosinophils and neutrophils recruitment while prostaglandin D2 release is responsible for persistence nasal obstruction.

In addition to these events, various cytokines and chemokines transcription are induced and released. Among them are IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-13, TNF-α, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 (MIP-1), granulocyte macrophage colony-stimulating factor (GMCSF) and regulated upon activation normal T expressed and secreted (RANTES) (Gleeson *et al*, 2008).

This inflammation process that occurs in allergic rhinitis also has been linked to the excessive production of Th2 cytokines interleukin IL-4 and IL-5 relative to Th1 cytokine interferon INF-γ and IL-10. (De Wall *et al*, 1991; Fiorentino *et al*, 1991). These two types of Th1 cytokines act as a regulatory or suppressor cytokine and has an important role in regulating the allergic immune response by facilitating T-cell tolerance and prevention of tissue inflammation.(Belinghausen *et al*, 2001; Takanashi *et al*, 1999).

1.2.3 Late Phase Reaction

After experiencing the early phase response, many patients show a late phase reaction characterized by cell adhesion molecule overexpression, release of inflammatory mediators with chemotactic activity and cytokine production that result in local tissue reaction (Naclerio *et al*, 1985; Skoner, 2001). It happens several hours after the exposure. At this phase, allergic sufferers might complain of nasal congestion, fatigue, malaise and irritability (Skoner, 2001).

Various effector cells involved for local tissue reaction in this phase. Mainly, they are eosinophils, basophils, mast cells, T lymphocytes, neutrophils and macrophages. Under the effect of chemotactic factors and modulating adhesion molecules that derived from endothelial and epithelial cells, these circulating effector cells adhere to the vascular endothelium and transmigrate to the reaction site. Cytokines levels also increase in this phase, especially IL-1, IL-4, IL-5, IL-6, IL-8, GM-CSF, soluble intercellular adhesion molecule-1 (ICAM-1) and TNF-α. (Flint *et al*, 2010).

Eosinophils play an important role in this stage. Tissue migration and activation of this effector cells are mediated and regulated by IL-5, GM-CSF, leukotriene B_4 (LTB_4), ICAM, vascular cellular adhesion molecule (VCAM) and several other chemokines such as RANTES and eotaxin (Gleeson *et al*, 2008).

Once eosinophils mature in nasal mucosa, they secrete major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), peroxidase and beta glucoronidase. They also have the ability to produce and release cytokines to cause further allergic inflammatory reaction. Among them are IL-3, IL-5, GM-CSF, cysteinyl leukotrienes, prostaglandin E1 and transforming growth factor (TGF) β1. All of these causing increase vascular permeability, mucus secretion and further recruitment of effector cells.

1.2.4 Systemic Activation

In final phase, systemic activation of immune processes can be observed in patient with allergic rhinitis. A stable basophilia is one of the effects of systemic activation. Circulating basophils could increase up to 2 folds during allergy season (Bochner, 2000). Since they are the only leukocytes that contain histamine in the blood stream, individuals with allergic rhinitis would have a larger source of histamine to be released during allergic response. Studies also have shown that circulating basophils are the main source for IL-4 and IL-13 production in response to specific antigen (Bochner, 2000).

There would also be an increased eosinophils numbers in the bone marrow, blood stream and tissues. One of the specific eosinophil growth factor, IL-5 which is secreted by Th2-cell is responsible for the increase production of eosinophils in bone marrow. From the bone marrow, eosinophils travel in circulation to reach the site of allergic inflammation and responsible for inflammatory reaction as mentioned. It has been observed that on initial event of allergen inhalation there was a decrease in the level of eosinophil in circulation followed by a rebound increase. The assumption for the initial decrement is the migration of eosinophils from the circulation to the site of inflammation and followed by increase production and release of eosinophils from bone marow under the influence of IL-5 (Bochner 2000).

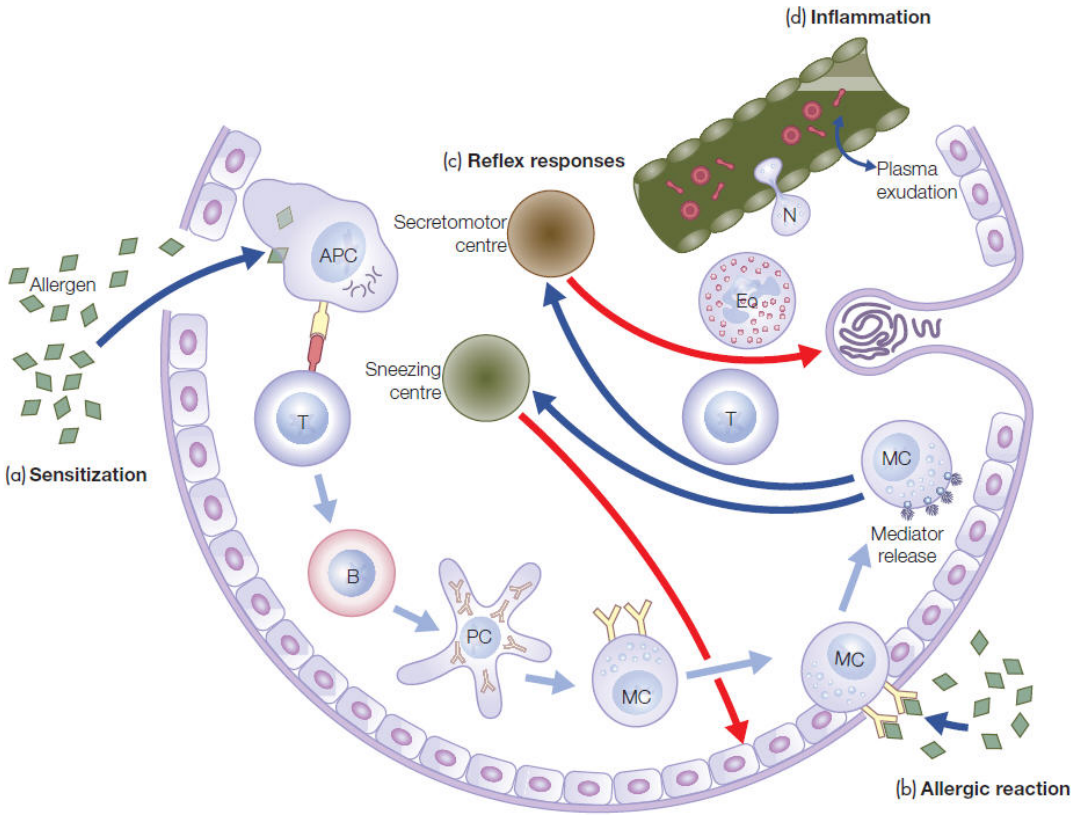

Figure 1: Phases of allergic reaction in the nose (Adapted from Gleeson M *et al.* 2008).

1.3 Eosinophil Count in Allergic Rhinitis

In allergic rhinitis, the skin prick test and specific IgE give the best predictive value. However serum inflammatory markers such as total serum IgE, eosinophil cationic protein and eosinophil percentage still can be the strong predictor of allergic rhinitis as reported by Jung and colleagues (Jung *et al*, 2011). They concluded the cut-off values in serum sample for these markers as followed - 98.7 IU/ml for IgE, 24.7µg/ml for eosinophil cationic protein (ECP) and 4.0% for eosinophils count. The specificity of eosinophils count for allergic rhinitis that was concluded in the study is 72%.

Eosinophil count is related to severity of allergic rhinitis. To compare the effectiveness of alternative medicines and herbs in treating allergic rhinitis, various studies used eosinophil counts as a parameter (Chen *et al*, 2009; Kalus *et al*, 2003; Xiao *et al*, 2006; Yang *et al*, 2010).

However it is uncommon for eosinophil count to exceed 1500/ul in allergic rhinitis (Nutman, 2007). High eosinophil level in serum or eosinophilia also can be observed in other illnesses such as parasitic infection, atopic dermatitis, malignancy, fungal infection, drug intake and certain immunologic and endocrine disorders. Eosinophil level in the blood stream also has its normal physiological fluctuation with the highest level in peripheral blood in the morning (Nutman, 2007).

1.4 Interleukin-4 in Allergic Rhinitis

In the individual with allergic rhinitis, interleukin-4 (IL-4) is increase in serum and nasal lavage. IL-4 plays a central role in the IgE synthesis system, the development of Th-2-like cells, and co-ordination as well as the persistence of airway inflammatory process in allergic disorders. Treatment with anti-histamine and immunotherapy will decrease it level.

The percentage decrease in IL-4 is correlated significantly with the percentage decrease in specific IgE antibodies following long-term immunotherapy (Ohashi *et al*, 1996). In a study, to observe the effect of antihistamine type-2 on serum interferon gamma, IL-4 and

IL-12 in allergic rhinitis patients, it has been shown that immunotherapy and H2 receptor antagonist lead to improvement in symptoms with the decrease of IgE antibodies and IL-4 in blood (Testa *et al,* 2001). While in another observation following usage of a conventional anti-histamine, cetrizine, an increase of IFN-γ/IL-4 ratio was observed (Uğuz *et al*, 2005).

In alternative treatment for allergic rhinitis, Li *et al* used allergic rhinitis models in rats to investigate the effect of garlicin on serum level of INF-gamma and IL-4. They had shown a significant decrease in serum level IL-4 together with increment of serum INF- γ after garlicin administration (Li *et al,* 2008).

1.5 Total Nasal Symptoms Score

Total Nasal Symptoms Score is the sum of all four nasal symptoms score. The score is based on four main nasal symptoms of allergic rhinitis which are nasal obstruction, nasal itchiness, rhinorrhea and nasal itching. It is measured on four point rating scale for each symptom. Until now, there was no single universally accepted scale to rate allergic rhinitis symptoms, however this four point rating scale is generally accepted. According to European Medicines Agency Guideline and U.S Food and Drug Administration for clinical development program of allergic rhinitis, the scale that were used are as follows (Himmel, 2000) :

- 0 = absent of symptoms (no sign/symptoms evident)

- 1 = mild symptoms (sign/symptoms clearly present, but minimal awareness, easily tolerated)
- 2 = moderate symptoms (definite awareness of sign/symptom that is bothersome but tolerable)
- 3 = severe symptoms (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping)

1.6 *Channa striatus*

Snakehead fish (*Channa striatus*, Channidae), belongs to the family Channidae. It exists as a wild freshwater, carnivorous fish commonly found in shallow water. It is widely distributed in Malaysia, South-East Asia countries, Taiwan, India and Indochina region (Mohsin & Ambak, 1983). It is native to Malaysia and has been proved it existed in our region since 600,000 years ago (Mat Jais, 2007).

Traditionally, it has been used for generations in post-partum mother and post-trauma patient. Among Malay, Chinese and Indian community, it is believed that this fish is a great natural remedy. It has been consumed as a protein source in their diet or prepared specifically for harnessing their healing potential. In some region, it is not a weird scene when the living fish is swallowed and was claimed that it gives a miraculous effect to their illness (BBC, 2003).

Because of this vast potential, since the past twenty years researchers have done extensive studies and proved that this fish in deed has great medicinal properties. It contains 17 essential amino acids and has high lipid content (Zakaria *et al*, 2007). The amino acids that present in high amount in this fish is glutamic acid, aspartic acid and lysine (Zuraini *et al*, 2005). The lipid contains mostly various kinds of essential fatty acids. It contains rich amount of docosahexaenoic acid (DHA), oleic acid, stearic acid and arachidonic acid (Zuraini *et al*, 2005). These essential fatty acids that present in the fish have a role in modulating prostaglandin metabolism hence the inflammation process (Simopoulos, 2002).

Apart from its antinociceptive properties (Mat Jais *et al*, 1997) and ability to promote wound healing (Baie & Sheikh, 2000), a study by Zakaria 2008 showed that *Channa striatus* fillet aqueous and lipid-based extracts of the fish have significant antiinflammatory activity. They performed experiment on rats using the haruan extract and gave evidence of its antiinflammatory activity. In his study, antiinflammatory and antipyretic activities were assessed using the carrageenan-induced paw edema and brewer's yeast-induced pyrexia tests. The aqueous and lipid-based extracts were found to show significant antiinflammatory activity (Zakaria, *et al*, 2008).

Channa striatus extract usage also shown a reduction in inflammation of osteroarthritic rabbits joint. In this preliminary study, inflammation of arthritic joints was reduced in joints treated with *Channa striatus* (Michelle *et al*, 2004).

Anti-inflammatory action of *C.striatus* extract could be explained by the high concentration of its fatty acids content. Stearic acid and oleic acid in the extract could suppress inflammatory responses by attenuating the polymorphonuclear leucocytes activity (Zakaria *et al*, 2007). The presence of high proportion of monosaturated fatty acid such as oleic acid in plasma could lead to decrement of arachidonic acid proportion in plasma. As we know from arachidonic acid metabolism (Fig. 2), inhibition of its cascade will result in reduction of eicosanoids formation hence decrease inflammation (Croker *et al*, 2002).

Another possible explanation for its anti-inflammatory action is the role of its DHA content. DHA could alter the arachidonic acid metabolism and inhibits its proinflammatory lipid mediator's formation (Mat Jais, 2007).

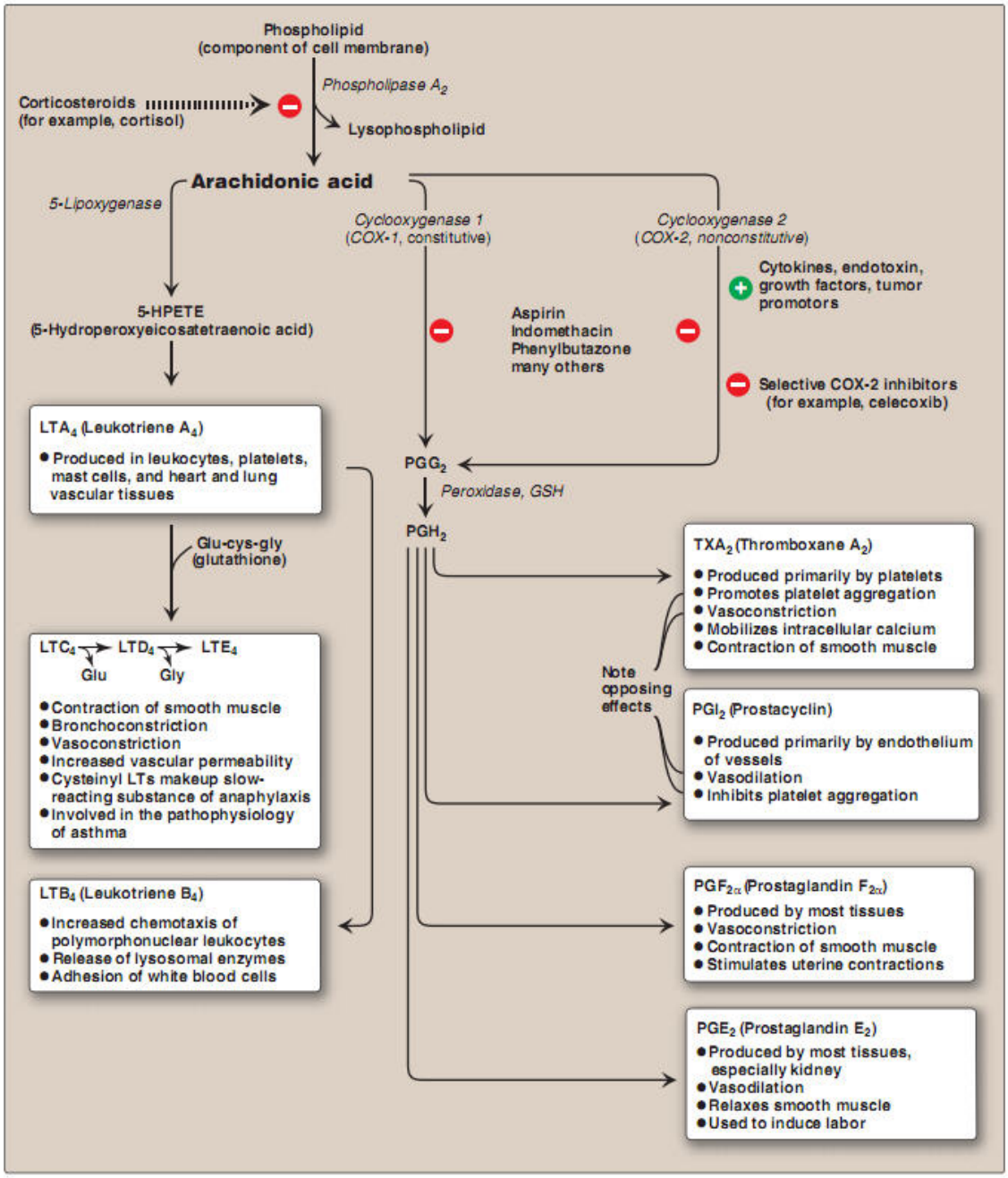

Figure 2: Biosynthesis of leukotrienes, prostaglandin and thromboxane from arachidonic acid (Adapted from Harvey R, Ferrier D., 2011. *Lippincott's Illustrated Reviews: Biochemistry, Fifth Edition.*)

1.7 Essential Fatty Acids and its role in Allergic Rhinitis

Since industrialization, the human diet has changed. People tend to eat more processed food that contains unbalanced composition of various fat. Studies in Europe suggested that their modern diet contains more polyunsaturated fatty acids which could be precursor to arachidonic acid. Excessive formation of arachidonic acid could lead into more formation of IgE in the body (Dunder *et al*, 2001). By taking more balanced diets that contains n-3 fatty acids, it could decrease the risk of allergic rhinitis and atopic diseases (Dunder *et al*, 2001, Hoff *et al*, 2005). N-3 fatty acids or commonly known as omega-3 fatty acids that involved in our physiology are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The main source of ALA in human is from plant origin. It is known that human body is not efficient in converting the ALA into EPA and DHA (Brenna, 2005). Hence, most of EPA and DHA that we need have to be derived from our diet. Among animals that are efficient to produce EPA and DHA are fish (Kris-Etherton *et al*, 2003).

In allergic rhinitis, leukotriene B_4 (LTB_4), one of the proinflammatory lipoxygenase products plays an important role in its pathophysiology. Its level in the blood is elevated in various allergic diseases. This leukotriene is produced by leukocytes from the membrane phospolipid arachidonic acid (Ohnishi *et al*, 2008). LTB_4 action in allergic rhinitis is related to its role in inducing IgE production by normal peripheral blood mononuclear cells and E-cells (PBMC partially depleted of T cells). However, it cannot react directly on the PBMC or E-cells alone. Its reaction depends on availability of Interleukin-4 (Yamaoko *et al*,

1994). LTB_4 also play its role in allergic rhinitis by becoming a chemo-attractant to the eosinophils (Kikuchi *et al*, 1998).

Studies showed that DHA and EPA that present in fish and fish oil has modulating effect on the eosinophil chemotactic and chemokinetic response to LTB_4 (Kikuchi *et al*, 1998). In human body, the ingested DHA and EPA will be integrated into neutrophil and monocytes cell membranes and change its arachidonic acid make-up, hence inhibit the LTB_4 formation from arachidonic acid (Ohnishi *et al*, 2008).

1.8 Rationale/Justification of Study

In today's modern world, around the globe more and more people tried to live as natural as possible. It gives alternative and complementary medicine a place in this modern world. Traditional medications resurfaced with more scientific evidences. As clinicians in a tropical country that has abundant natural resources with medicinal properties that can be exploited and researched further, we took this opportunity to explore the therapeutic potential that presents inside *Channa striatus* and its extract.

Through previous studies, we already knew that this fish has high protein and fatty acids content. Presence of various essential amino acids and essential fatty acids have made this fish capable to promote wound healing, modulating anti-inflammatory action and anti-nociceptive in our body.

Although various studies were done to investigate its anti-inflammatory action, there was no research conducted to study its anti-inflammatory potential in allergic rhinitis patients. We hope with the completion of this study, we can know its immune-modulatory effect or at least, its capability in relieving the symptoms of allergic rhinitis.

Our hope, this study will open a new path for further research in alternative therapy for allergic rhinitis sufferers. The success of this study indirectly will promote our local product to be used as an adjunct treatment, to be used as adjunct with the conventional treatment, and finally boosting our agricultural sector. Further research could be done to determine its optimal dosage in the future and its interaction with other dietary intake.

CHAPTER 2: OBJECTIVES

2.1 General Objective:

To determine the effect of *Channa striatus* extract as an adjunct treatment on the serum level of allergy inflammatory markers in allergic rhinitis patient.

2.2 Specific Objectives:

1. To determine the mean difference of serum eosinophil count for *Channa striatus* and placebo group before and after treatment.
2. To compare the mean difference of serum eosinophil count for *Channa striatus* and placebo group.
3. To determine the mean difference of serum Interleukin-4 between *Channa striatus* and placebo group before and after treatment.
4. To compare the mean difference of serum Interleukin-4 between *Channa striatus* and placebo group.
5. To observe the effect of *Channa striatus* extract as an adjunct treatment on patients' total nasal symptom scores.

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 Study Question

1. Does the *Channa striatus* extract intake will result in decrement of allergy inflammatory markers in patients with allergic rhinitis?

2. Does the *Channa striatus* extract will lead to decrease of total nasal symptoms score?

3.2 Study Hypothesis

Null hypothesis – There is no difference outcome in allergy inflammatory markers level between the *Channa striatus* extract group and the control group.

Alternative hypothesis – There is a significant decrement in serum allergy inflammatory markers in *Channa striatus* group compared to the control group.

3.3 Study Design

This is a double blind randomized controlled trial study of *Channa striatus* extract versus placebo in selected patients with allergic rhinitis.

3.4 Population and Sample

3.4.1 Subject Selection

The sampling population was recruited from patients who attended Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) clinic at Universiti Sains Malaysia Hospital, Kubang Kerian, Kelantan beginning from February 2014 till June 2014.

3.4.2 Sampling Method

Subjects were selected through convenience sampling. Patients who attended the ORL-HNS clinic and met the inclusion and exclusion criteria as mentioned below were included in the study.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria

1. Patients between 18-50 years old as more of older population suffering from non-allergic rhinitis.
2. Patients diagnosed as allergic rhinitis based on clinical examination.
3. Patients with skin prick test positive for allergy.
4. Patients with intranasal corticosteroid as main treatment.

3.5.2 Exclusion Criteria

1. Pregnancy and nursing females as it may affect the baby.
2. Patients with significant medical illness that on multiple medications.
3. Patients with severe atopic dermatitis, asthma and history of anaphylaxis.
4. Patient with concurrent rhinosinusitis or other nasal pathology.
5. Patients with history of nasal surgery.
6. Patients on systemic corticosteroid therapy.
7. Patients treated with systemic adrenergic drug.
8. Patients who have history of allergy to *Channa striatus* or its related product.
9. Patients who already took haruan as their regular diet.

3.6 Sample Size Calculation

The sample size was calculated using two means formula, based on previous study by Maiti *et al*, (2010).

We planned a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. In a previous study the response within each subject group was normally distributed with standard deviation

205.66. If the true difference in the experimental and control means is 166.9, we will need to study 23 experimental subjects and 23 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

$$n = \frac{2\sigma^2}{\Delta^2}(Z\alpha + Z\beta)^2$$

$$n = \frac{2\,(205.66)^2}{166.90^2}(1.96 + 0.84)^2$$

$$n = 23.3$$

Hence, 23 subjects for each group included twenty percent calculated drop-out, with total of 56 subjects as the sample size.

3.7 Study Protocol and Ethical Consideration

3.7.1 Study Protocol

The study protocol was approved by Research Ethics Comittee (Human), Universiti Sains Malaysia, Kubang Kerian, Kelantan [Ref. no: USM/JEPeM/273.3(9)]. Patients who

attended ORL-HNS clinic and fulfilled the inclusion and exclusion criteria were recruited for this study. After a detailed explanation regarding the diagnosis and proposed treatment, patients were allowed to make decision either want to continue their conventional medication or volunteer to join the study and using the alternative treatment as an adjunct medication. Risk and benefit of the studied medication explained. An informed written consent was obtained from each patient who participated in this study. Patients underwent routine history taking, clinical assessment and skin prick test for allergic rhinitis. After randomization, they were divided into study and control group. Blood samples for FBC, RFT, LFT, differential count for eosinophil and IL-4 were taken at initial visit and after 6 weeks of treatment. At 2 weeks of treatment, a clinical assessment was made to monitor patient compliance or any adverse effects that may occur. Collected data then analyzed using computer software.

3.7.2 Ethical Consideration

After thorough explanation, the well informed patients were asked to sign the written consent. A copy of the written and signed consent was given to each participant. All participants in this study involved voluntarily and they were permitted to withdraw from the study at any moment they considered necessary.

3.8 Methods and Instruments

3.8.1 Methods

Patients who attended the clinic with allergic rhinitis symptoms were seen by investigator and diagnosis were made based on history and physical examination. Informed consents were taken from the patients. Those who agreed to participate in the study were subjected to skin prick test.

Subject who met the criteria of allergic rhinitis and had positive skin prick test was included in the study. Those who was on oral anti-histamine was asked to stop taking their medication at least two weeks prior to participation in this study as a wash-out period while still continuing using intranasal corticosteroid. For those who has consumed haruan regularly or taking traditional medication, they were asked to withhold their intake 1 month prior to the study.

Patients were subjected to 2 follow-ups at week 2 and week 6 after the treatment initiated. They began with answering questionnaire related to their symptoms and were given the trial drug. The questionnaire was intended to assess their total nasal symptoms score. Until now, there were no single universal symptoms score for allergic rhinitis accepted as a standard. However the measurement based on 4 point rating scale is widely accepted. The nasal symptoms that were assessed are nasal obsctruction, nasal discharge/rhinorrhea,