



UNIVERSITI PUTRA MALAYSIA

**ANTI-INFLAMMATORY ACTIVITY OF HARUAN
(*Channa striatus* Bloch) CREAM ON
12-0-TETRADECANOYLPHORBOL-13-ACETATE-INDUCED
CHRONIC DERMATITIS IN MICE MODEL**

IRMA IZANI MOHAMAD ISA



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12-0-TETRADECANOYLPHORBOL-13-ACETATE-INDUCED
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By

IRMA IZANI MOHAMAD ISA

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the
Requirements for the Degree of Master of Science**

September 2015



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I dedicate this piece of work to my wonderful husband, Abdu Syakur bin Hamid and my beloved children, Abdul Basit and Nusaybah, who have been my source of inspiration and strength.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the degree of Master of Science

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September 2015

Chairman : Professor Abdul Manan Mat Jais, PhD
Faculty : Medicine and Health Sciences

Haruan or *Channa striatus* has been traditionally well-known for its pharmacological benefits in wound healing. Atopic dermatitis or eczema remains a challenge globally due to difficulty in treating the condition, the side effects of corticosteroid-based treatment and the increasing prevalence of the disease worldwide including Malaysia. This research was aimed to discover anti-inflammatory effect of Haruan cream, made from the fish extract on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced chronic-like dermatitis model on mice ears, by analysing ear oedema, histological findings and gene expression of inflammatory cytokines including TNF- α , IL-1 β , IL-10 and IL-4. Briefly, both surfaces of mice ears were applied with 10 μ l of TPA (2.5 μ g/ 20 μ l acetone) for five times on alternate days (day 0, 2, 4, 7 and 9) and with Haruan cream (HC 1%, HC 5% or HC 10%) to the same site twice daily for the last three days of the treatment course (day 7, 8 and 9). When measured on day 8 or 24 hours after the first treatment with Haruan cream, reduction of thickness in mice ear was not statistically significant as compared to negative control. Whereas, when measured on day 10 or 24 hours after the final treatment with Haruan cream, mice ear thickness was significantly reduced ($p < 0.05$) to 0.547 ± 0.025 mm (19.44%) in TPA+HC 1%, 0.556 ± 0.018 mm (18.11%) in TPA+HC 5% and 0.489 ± 0.015 mm (28%) in TPA+HC 10%, in comparison to negative control. Haruan cream also had produced comparable effect as to positive control, hydrocortisone 1% (H-Cort) which has ear thickness of 0.557 ± 0.022 mm or 18% reduction from negative control. Histological comparison had showed evident reduction in various indicators of cutaneous inflammation including dermal oedema, inflammatory cell infiltration and proliferation of epidermal keratinocytes upon treatment with Haruan creams. Gene expression analysis using RT-qPCR method had showed that TNF- α was downregulated from 352.75-fold in negative control to 34.36-fold, 54.19-fold and 112.40-fold in TPA+HC 1%, TPA+HC 5% and TPA+HC 10% groups respectively, with significant reduction ($p < 0.05$) in both TPA+HC 1% and TPA+HC 5% as compared to negative control. However, there was no significant upregulation of IL-10 by Haruan creams as compared to negative control. Besides, no expression of IL-1 β and IL-4 was detected in this animal model of chronic dermatitis. In conclusion, *C. striatus* is an

effective anti-inflammatory agent in this murine phorbol ester-induced chronic dermatitis model, thus suggesting its potential to treat various chronic inflammatory skin diseases including atopic dermatitis.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**AKTIVITI ANTI-RADANG KRIM HARUAN
(*Channa striatus* Bloch) KEPADA RADANG KULIT KRONIK
ARUHAN 12-O-TETRADECANOYLPHORBOL-13-ACETATE
DALAM MODEL MENCIT**

Oleh

IRMA IZANI MOHAMAD ISA

September 2015

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Haruan atau *Channa striatus* telah diketahui secara tradisi mempunyai faedah farmakologi dalam penyembuhan luka. Radang kulit atopik atau ekzema terus menjadi cabaran di peringkat global kerana penyakit ini sukar diubati, kesan sampingan daripada rawatan berasaskan kortikosteroid dan peningkatan kes penyakit ini di serata dunia termasuk Malaysia. Kajian ini bertujuan untuk mengkaji kesan anti-radang krim Haruan yang diperbuat daripada ekstrak ikan Haruan pada model radang kulit kronik mencit teraruh 12-O-tetradecanoylphorbol-13-acetate (TPA), dengan mengkaji edema pada telinga, perubahan histologi dan ekspresi gen sitokin keradangan termasuk TNF- α , IL-1 β , IL-10 dan IL-4. Secara ringkasnya, kedua-dua permukaan telinga mencit disapukan dengan 10 μ l TPA (2.5 μ g/ 20 μ l aseton) untuk lima kali selang sehari (hari ke 0, 2, 4, 7 dan 9) dan disapu krim Haruan (HC 1%, HC 5% atau HC 10%) dua kali sehari pada tempat yang sama pada tiga hari terakhir rawatan (hari ke 7, 8 and 9). Ketika diukur pada hari ke-8 atau 24 jam selepas rawatan pertama dengan krim Haruan, penurunan ketebalan telinga mencit adalah tidak signifikan secara statistik berbanding kawalan negatif. Sebaliknya, ketika diukur pada hari ke-10 atau 24 jam selepas rawatan terakhir dengan krim Haruan, ketebalan telinga mencit telah menurun dengan signifikan ($p < 0.05$) kepada 0.547 ± 0.025 mm (19.44%) dalam TPA+HC 1%, 0.556 ± 0.018 mm (18.11%) dalam TPA+HC 5% dan 0.489 ± 0.015 mm (28%) dalam TPA+HC 10% berbanding kawalan negatif. Krim Haruan juga menghasilkan kesan yang serupa dengan kawalan positif, hydrocortisone 1% (H-Cort) yang mempunyai ketebalan telinga 0.557 ± 0.022 mm atau 18% penurunan berbanding kawalan negatif. Perbandingan histologi menunjukkan bukti penurunan untuk pelbagai indikasi radang kulit termasuk oedema pada lapisan dermis, penyusupan sel radang, dan proliferasi keratinosit pada lapisan epidermis selepas rawatan dengan krim Haruan. Analisis ekspresi gen dengan menggunakan kaedah RT-qPCR menunjukkan pengekspresan TNF- α telah menurun daripada 372.75 ganda kepada 34.36-ganda, 54.19-ganda dan 112.40-ganda masing-masing untuk kumpulan TPA+HC 1%, TPA+HC 5% dan TPA+HC 10%, dengan penurunan pengekspresan yang signifikan ($p < 0.05$) pada TPA+HC 1% and TPA+HC 5% berbanding kawalan negatif. Sebaliknya, tiada peningkatan yang signifikan terhadap pengekspresan IL-10 oleh krim Haruan berbanding kawalan negatif. Selain itu,

pengekspresan IL-1 β and IL-4 tidak dapat dikesan dalam model haiwan radang kulit kronik ini. Kesimpulannya, *C. striatus* adalah agen anti-radang yang efektif pada model radang kulit kronik mencit teraruh phorbol ester, seterusnya menunjukkan potensinya dalam merawat pelbagai penyakit radang kulit kronik seperti radang kulit atopik.



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I certify that a Thesis Examination Committee has met on 29 September 2015 to conduct the final examination of Irma Izani Mohamad Isa on her thesis entitled “Anti-Inflammatory Activity of Haruan (*Channa striatus* Bloch) Cream on 12-0-Tetradecanoylphorbol-13-Acetate-Induced Chronic Dermatitis in Mice Model” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

| | | |
|-----------------------|---|--|
| $\Delta\Delta C_q$ | - | Comparative |
| ANOVA | - | analysis of variance |
| APCs | - | antigen-presenting cells |
| c-MAF | - | c-musculoaponeurotic fibrosarcoma |
| COX-1 | - | cyclooxygenase 1 |
| COX-2 | - | cyclooxygenase 2 |
| C_q | - | quantification cycle |
| DHA | - | docosahexaenoic acid |
| DPX | - | Di-N-Butyle Phthalate in Xylene |
| EPA | - | eicosapentaenoic acid |
| FLG | - | filaggrin gene |
| GAP | - | Good Agricultural Practices |
| GAPDH | - | glyceraldehyde-3-phosphate-dehydrogenase |
| GM-CSF | - | granulocyte-macrophage colony-stimulating factor |
| H&E | - | hematoxylin and eosin |
| HC | - | Haruan cream |
| H-Cort | - | Hydrocortisone |
| HM | - | Haruan Manan |
| HPA | - | hypothalamic-pituitary-adrenal axis |
| HTE | - | Haruan Traditional Extract |
| IACUC | - | Institutional Animal Care and Use Committee |
| ICAM-1 | - | intercellular adhesion molecule 1 |
| IFN- γ | - | interferon γ |
| IgE | - | immunoglobulin E |
| I κ B | - | inhibitor of kappa B |
| I κ B α | - | inhibitor of kappa B alpha |
| IKK | - | inhibitor kappa B kinase |
| IL-1 | - | interleukin 1 |
| IL-6 | - | interleukin 6 |
| iNOS | - | inducible nitric oxide synthase |
| LOX | - | lipoxigenase |
| MAPK | - | mitogen-activated protein kinase |
| MCP-1 | - | monocyte chemotactic protein 1 |
| MIQE | - | Minimum Information for Publication of Quantitative Real- Time PCR Experiments |
| MPO | - | myeloperoxidase |
| NF- κ B | - | nuclear factor kappa B |
| NK | - | natural killer cells |
| NTC | - | no template control |
| PC12 | - | phaeochromocytoma 12 |
| PGE2 | - | prostaglandin E2 |
| PGG2 | - | prostaglandin G2 |
| PGH2 | - | prostaglandin H2 |
| PGP 9.5 | - | protein gene product 9.5 |
| PKC | - | protein kinase C |
| PLA2 | - | phospholipase A2 |
| PMNs | - | polymorphonuclear neutrophils |
| PUFA | - | polyunsaturated fatty acids |
| RIN | - | RNA integrity number |

| | | |
|---------------|---|---|
| RT-qPCR | - | real time reverse transcription polymerase chain reaction |
| SCID | - | severe combined immunodeficiency |
| SD | - | standard deviation |
| SEM | - | standard error of mean |
| STATs | - | Signal Transducers and Activators of Transcription |
| TE | - | Tris - ethylenediaminetetraacetic acid |
| Th1 | - | type 1 helper T cell |
| Th2 | - | type 2 helper T cell |
| TNF- α | - | tumour necrosis factor α |
| TPA | - | 12-O-tetradecanoylphorbol-13-acetate |
| TSLP | - | thymic stromal lymphopoietin |
| UPM | - | Universiti Putra Malaysia |



CHAPTER 1

INTRODUCTION

1.1 Background of Study

Inflammation is the first protective response to tissue injury or insult which aimed to remove injurious stimuli and to initiate the healing process. This process involves a complex interaction of a number of inflammatory mediators that include prostaglandin, nitric oxide, cytokines and many others that regulate the inflammatory responses. Acute inflammation is known for its cardinal signs of redness (rubor), swelling (tumor), heat (calor), pain (dolor) and loss of function (functio laesa) (Ryan & Majno, 1977). It is characterised by a series of events that begin with the increased blood flow and vascular permeability that subsequently followed by the infiltration of polymorphonuclear neutrophils (PMNs) and mediators. Whereas, chronic inflammation is associated with a prolonged duration of inflammation which comprises of both tissue destruction and repair together with accumulation of a different set of inflammatory cells including lymphocytes and macrophages.

Atopic dermatitis is a chronic relapsing inflammation of the epidermis layer of the skin characterised by skin dryness, pruritus, erythema, crust, excoriation and lichenification (Leung & Bieber, 2003). The term atopic dermatitis and eczema often used interchangeably to describe skin inflammation but atopic dermatitis is specifically referred to an atopic disease where patients or their family may have history of asthma and/or allergic rhinitis. While the aetiology of eczema remains puzzling, it is believed that the pathogenesis is multifactorial and involves complex interrelation of genetic, environmental and immunological factors (Leung, 2000). The traditional theory of immunological aberrations and the later impaired skin barrier had been the two proposed pathogenesis of this skin disorder. Besides, the increased production of inflammatory cytokines and cyclooxygenase-2 (COX-2) derived prostanoids including prostaglandin E2 (PGE2) are thought to play a major role in the pathogenesis of the disease (Honda *et al.*, 2010; Elias *et al.*, 1999).

Treatment strategies for atopic dermatitis has included a combination of pharmacological and non-pharmacological approaches. Avoidance of irritants and allergens, maintaining good skin hydration and the use of topical corticosteroids and immunomodulatory agents are the three fundamentals in the management of atopic dermatitis (Chang *et al.*, 2007). The key element in care of eczema is identification and avoidance of triggering factors which include soaps, detergents and some food like egg and cows' milk (Shams *et al.*, 2011). Beside the use of emollient and topical corticosteroids, natural and alternative treatments has contributed greatly and commonly use now in treating eczema (Chang *et al.*, 2007).

Haruan *Channa striatus* has been traditionally well-known for its natural benefit in wound healing. Known as Haruan among the Malays, it has been a popular fish in Malaysia as a source of protein but is more commonly consumed by post-partum women and post-surgical patients to accelerate wound healing. It is a snakehead, tropical freshwater fish that commonly found in Asian countries including India, China, Malaysia and Thailand (Mohsin & Ambak, 1983). In the recent years, more scientific studies had proven other pharmacological values of the fish including its anti-inflammatory, antinociceptive, antidepressant and neuroregenerative properties (Mohd Shafri & Mat Jais, 2012). These therapeutic effects of *C. striatus* are believed to be attributed by its good profile of fatty acids including DHA (docosahexaenoic acid) as well as high concentration of important amino acids (Zuraini *et al.*, 2006).

With regards to anti-inflammatory property of *C. striatus*, recently, Haruan in cream formulation had been proven to effectively reduce acute inflammation in mouse ear oedema model (Abedi *et al.*, 2012). On the other hand, this study had been designed to further demonstrate anti-inflammatory action of Haruan on chronic skin inflammation model induced by multiple application of 12-O-tetradecanoylphorbol-13-acetate (TPA) as proposed by Stanley and Steiner (1991). This model of chronic-like dermatitis produces a longer-lasting inflammation suitable for the evaluation of anti-inflammatory action of various substances on inflammatory mediators including cytokines and chemokines (Wang & Smart, 1999).

1.2 Problem Statement

As the most common type of inflammatory skin diseases, atopic dermatitis remain a challenge globally due to the reportedly increasing prevalence and estimated to affect around 10-20% of the population, especially infants and school-age children (Kim, 2013). In Malaysia, it is estimated that around 10-14% of children particularly are affected by eczema (Quah *et al.*, 2005). In fact, eczema may have an impact on quality of life due intense itch and other discomforts that may lead to psychological stress (Boehm *et al.*, 2012). Unfortunately, atopic dermatitis is a difficult disease to control and is still a treatment challenge (Chang *et al.*, 2007). To make things worse, the safety of using steroid-based medicaments for atopic dermatitis are doubted and has been the major concern among parents or carers who are conscious about the overwhelming side effects of the prolonged use of topical corticosteroids including skin irritation and atrophy (Hengge *et al.*, 2006)

1.3 Significance of Study

The significance of this study will be the discovery of the potential of *C. striatus* as natural product to be an effective, non-steroidal and safe treatment for various skin disorders including atopic dermatitis. While new treatment modalities will bring significant contribution in the treatment of atopic dermatitis, recent studies have failed to identify such a product (Chang *et al.*, 2007). Therefore, more studies should be encouraged to explore therapeutic effects of natural resources particularly Haruan. Moreover, research to produce a safe yet effective alternative treatment for eczema is necessary as treatment with steroid-based products are often less tolerated (Hengge *et al.*, 2006). Therefore, the outcome of this research is important to promote Haruan cream

as non-steroidal and effective treatment option for the management of eczema and other inflammatory skin diseases. Furthermore, in the effort to elucidate the mechanism of action of Haruan as anti-inflammatory agent, this study provides further evidences of anti-inflammatory property of Haruan by means of gene expression of inflammatory cytokines which play key role in the regulation of inflammatory responses.





1.4 Objectives

The main objective is to evaluate anti-inflammatory activity of Haruan cream on TPA-induced mouse model of chronic dermatitis.

Specific objectives of this study are:-

- a) To assess oedema in the mice ear by measuring ear thickness changes in TPA-induced chronic dermatitis model.
- b) To identify histological changes of cutaneous inflammation parameters in TPA-induced chronic dermatitis model.
- c) To establish gene expression studies of inflammatory-associated cytokines including TNF- α , IL-10, IL-1 β and IL-4 in TPA-induced chronic dermatitis model.

1.5 Hypothesis

Haruan is an effective anti-inflammatory agent on this mouse model of chronic skin inflammation as demonstrated by the reduction of ear oedema, the reduction of histological signs of cutaneous inflammation and the downregulation of pro-inflammatory and the upregulation of anti-inflammatory cytokines.

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