



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF FLAVOKAWAIN A ON INTERLEUKIN-6 AND MELANIN
PRODUCTIONS IN HISTAMINE-INDUCED HaCaT KERATINOCYTES
CELLS AND MOUSE B16-F10 MELANOMA CELLS***

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan projek bertajuk “ **Effects of Flavokawain A on Interleukin-6 and Melanin Productions in Histamine-induced HaCaT Keratinocytes Cells and Mouse B16-F10 Melanoma Cells**” telah disediakan serta dikemukakan kepada Jabatan Biokimia oleh **KIM JI MAN** sebagai syarat untuk kursus BCH 4999 projek.

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LISTS OF ABBREVIATIONS

2-S-CD	: 2-S-cysteinyl-dopa
5-S-CD	: 5-S-cysteinyl-dopa
6-S-CD	: 6-S-cysteinyl-dopa
AC	: Adenylyl cyclase
AD	: Atopic dermatitis
cAMP	: cyclic adenosine monophosphate
COX-2	: Cyclooxygenase-2
CRE	: cAMP-responsive elements
CREB	: cAMP-responsive-element binding
DAG	: Diacylglycerol
DCT	: Dopachrome tautomerase
DHI	: Dihydroxyindole
DHICA	: Dihydroxyindole-2-carboxylic acid
DQ	: Dopamine quinone
ERK	: Extracellular signal-regulated kinases
ET-1	: Endothelin-1
FLA	: Flavokawain A
H1R	: Histamine H1 receptor
IKK	: I kappa B kinase
L	: Litre
IL-1 α	: Interleukin-1 α
IL-1	: Interleukin-1
IL-1 β	: Interleukin-1 β
IL-6	: Interleukin-6
IL-8	: Interleukin-8

IP ₃	: Inositol-1,4,5-trisphosphate
LPS	: Lipopolysaccharide
LTB ₄	: Leukotriene B ₄
MAPK	: Mitogen-activated protein kinase
MC1R	: Melanocortin-1 receptor
mg	: Milligram
μg	: Microgram
ml	: Millilitre
NF-κB	: Nuclear factor- kappaB
NO	: Nitric oxide
PGD ₂	: Prostaglandins D ₂
PGE ₂	: Prostaglandin E ₂
PIH	: Post-inflammatory hyperpigmentation
PKA	: Phosphokinase A
PKC	: Phosphokinase C
PLC	: Phospholipase C
TNF-α	: Tumor necrosis factor-alpha
TYR	: Tyrosinase
TYRP 1	: Tyrosinase-related protein 1
α-MSH	: Alpha melanocyte stimulating hormone

ABSTRACT

Chronic skin inflammatory diseases affecting large populations in Malaysia and this condition is getting serious globally. Post-inflammatory hyperpigmentation (PIH) is one of chronic skin inflammatory diseases which usually resulted after inflammation process raises health, cosmetic and psychosocial concerns. Resultantly, anti-inflammatory and skin depigmenting agents have been developed to alleviate the effects thereof. However, current treatments on these diseases have potential toxicity and carcinogenicity. Therefore, there is a need to develop new anti-inflammatory and skin depigmenting agents with minimal side effects and greater efficacy. Chalcone derivatives is a group of compound belong to flavonoid family and has been reported with anti-inflammatory and anti-melanogenic properties. Thus study ought to determine the anti-inflammatory and anti-melanogenic effects of the chalcone compound 1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (Flavokawain A, FLA) on cytokine production (Interleukin-6) in 10 μM histamine-induced HaCaT cells and melanin content in 10 μM histamine-induced mouse B16-F10 melanoma cells. The results showed that 50 μM of FLA suppressed the IL-6 production by 44% with HaCaT cell viability of 75%. Nevertheless, 50 μM of FLA was successfully reduced the melanin content by 73% with the B16-F10 melanoma cell viability of 78%. FLA showed high inhibitory activity towards melanin production in histamine-induced B16-F10 melanoma cells. This has indicates that FLA possess potential anti-inflammatory and anti-melanogenic activities. Thus, FLA has shown potential anti-inflammatory and depigmenting activities *in vitro*. Therefore, FLA might has the potential to be developed as an agent or alternative medicine to treat PIH. However, further studies need to be done to evaluate its efficacy and safety.

ABSTRAK

Kulit radang penyakit kronik telah menjejaskan populasi yang besar di Malaysia dan keadaan ini juga menjadi semakin serius di seluruh dunia. Hiperpigmentasi yang berlaku selepas proses radang merupakan salah satu penyakit kulit radang kronik yang biasanya berlaku selepas proses keradangan dan hal ini telah menimbulkan kebimbangan terhadap kesihatan, kosmetik dan psikososial. Berikutan perkara tersebut, agen anti-radang dan penyahpigmen pada kulit telah dihasilkan untuk mengurangkan kesan-kesan tersebut. Walau bagaimanapun, rawatan semasa mengenai penyakit-penyakit ini mempunyai ketoksikan yang berpotensi dan kekarzinogenan. Oleh itu, terdapat keperluan untuk memajukan ejen penyahpigmen dan anti-radang kulit baru dengan kesan sampingan yang minimum dan keberkesanan yang lebih tinggi. Derivatif *Chalcone* adalah kumpulan sebatian berasal dari keluarga flavonoid dan telah dilaporkan dengan ciri-ciri anti-radang dan penyahpigmen. Oleh itu, kajian ini bertujuan untuk menentukan kesan anti-radang dan anti-melanogenik kompaun *chalcone* 1- (2-Hydroxy-4,6-dimethoxyphenyl) -3- (4-methoxyphenyl) prop-2-en-1-satu (Flavokawain A, FLA) mengenai pengeluaran *cytokine* (Interleukin-6) sel HaCaT yang telah dirangsang dengan 10 μ M histamine dan juga pengeluaran melanin pada sel B16-F10 melanoma yang telah dirangsang dengan 10 μ M histamine. Hasil kajian menunjukkan bahawa 50 μ M FLA telah mengurangkan pengeluaran IL-6 sebanyak 44% dengan kadar hidup sel HaCaT sebanyak 75% manakala 50 μ M FLA telah berjaya mengurangkan pengeluaran melanin sebanyak 73% dengan kadar hidup sel B16-F10 melanoma sebanyak 78%. FLA menunjukkan aktiviti perencatan terhadap pengeluaran melanin dalam sel B16-F10 melanoma yang telah dirangsang dengan histamine. Hal ini telah menunjukkan bahawa FLA mempunyai potensi aktiviti anti-radang dan anti-melanogenik *in vitro*. Oleh itu, FLA berpotensi untuk dimajukan sebagai agen atau perubatan alternatif untuk merawat PIH. Walau bagaimanapun, kajian lanjut perlu dilakukan untuk menilai keberkesanan dan keselamatan FLA.

CHAPTER 1

INTRODUCTION

Inflammation is a process in which an organism used to remove injurious stimuli and is the starting point of healing process in order to get rid of foreign particles (Marone *et al.*, 2003). However, prolonged inflammation will lead to chronic skin inflammatory diseases. In the inflammation process, histamine (β -imidazoleethylamine) act as inflammatory mediator (Mizuguchi *et al.*, 2012).

Atopic dermatitis (AD), lichen planus, psoriasis and allergic contact dermatitis are chronic skin inflammatory diseases that are commonly found (Lynde *et al.*, 2006). There are about 8% to 25% of the population worldwide being affected by AD, and the incidence is increasing, especially in developed countries (Weston and Howe, 2008).

During inflammation, various cytokines and chemokines will be released via NF- κ B pathway. This will results in transcription of pro-inflammatory cytokines genes like IL-6, IL-1 β and TNF- α . The continued releasing of these pro-inflammatory cytokines will lead to chronic skin inflammatory diseases. For example, IL-6 exerts stimulatory effects on T- and B-cells, thus favoring chronic inflammatory responses. So, strategies targeting IL-6 and IL-6 signaling led to effective prevention and treatment of models on chronic inflammatory diseases (Gabay, 2006).

In addition, hyperpigmentation are usually accompanied with inflammation and this condition can be explained by accumulation of histamine secreted from mast cells during inflammation and this will stimulate melanocytes to produce increased amounts of pigment (Ruzicka *et al.*, 1983) as well as increases tyrosinase activity in melanocytes

(Yoshida *et al.*, 2000). The hyperpigmentation that occurred after the inflammation process is known as post-inflammatory hyperpigmentation (PIH).

PIH is defined as an acquired condition with excess of pigment deposited in the skin attributed to preceding skin inflammatory disease and is occurs at the site of skin injury after the inflammation has subsided. This acquired excess of pigment can be attributed to various preceding disease processes that affect the skin like infections, allergic reactions, mechanical injuries, reactions to medications, phototoxic eruptions, trauma and inflammatory diseases such as lichen planus, lupus erythematosus, atopic dermatitis). The patches that occurred may results from both increase in melanin production by individual melanocytes as well as increase in total number of melanocytes (Mak, 2010). Although PIH is not life-threatening, but patients who consider it a social embarrassment may lowered confidence of individual and eventually affected their social life.

Topical corticosteroids remain as the mainstay of anti-inflammatory treatment, it showing efficacy in the control of both acute and chronic skin inflammation. However, it will induce various cutaneous adverse effects in locally applied sites, such as telangiectasia, skin atrophy, hypertrichosis, hypopigmentation, perioral dermatitis and atrophic cutaneous striae (Furue *et al.*, 2003).

In the case of hyperpigmentation, hydroquinone (HQ) or 1,4-dihydroxybenzene has been used successfully to treat many forms of epidermal hyperpigmentation like melasma, freckles and PIH (Hu *et al.*, 2009). It is a phenolic bleaching compound which is also the gold-standard therapy for PIH. However, long-term used of HQ will bring

about side effect like ‘hydroquinone halo’. This condition is characterized by a ‘halo’ which surrounding the dark macule due to the bleaching of the surrounding normal skin (Woolery-Lloyd and Kammer, 2011). Besides that, there is report on patients who develop exogenous ochronosis (EO) on area where HQ has been applied although it was in low concentrations, (2%) and for short periods of time (6 months) (Ribas *et al.*, 2010). Concern regarding the side effects of HQ has arisen because the benzene metabolites of hydroquinone *in vivo* also seem to be involved in bone marrow toxicity and even carcinogenesis when extensively used (Levitt, 2007; Nordlund *et al.*, 2006). Moreover, hydroquinone also considered to be cytotoxic to melanocytes (Briganti *et al.*, 2003; Hermanns *et al.*, 2000).

In recent years, extensive researches have been done for development of more effective and safer drugs in the treatment of chronic skin inflammatory skin disease as well as the alternative treatment on PIH. One of the bioactive compounds that researchers interested in is chalcones. Chalcones are compounds that mostly found in plants and its anti-inflammatory and anti-melanogenic characteristics maybe playing role in inflammation process and melanogenesis and thus might be an alternative to cure chronic skin inflammatory disease and PIH. Furthermore, chalcones are abundant in edible plants (Nowakowska, 2007) and this is believed to have less side effects than the current medication in treating of both chronic skin inflammatory disease and PIH.

Inhibition of cytokines production by chalcones could be an important therapeutic intervention in skin inflammatory diseases and this may lead to the discovery of new drugs as alternative in treating chronic skin inflammatory disease and PIH.

The objectives of this study were :

1. To evaluate the effects of synthesized chalcones 1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (FLA) on cytokines production (Interleukin-6) in histamine-induced HaCaT keratinocytes cells.
2. To evaluate the effects of synthesized chalcones 1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (FLA) on melanin production in histamine-induced B16-F10 murine melanoma cells.

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