A STUDY ON THE GENE EXPRESSION LEVEL IN HaCaT KERATINOCYTE CELLS TO RELATE WITH HALAL AND HARAM STATUS WHEN EXPOSED TO PLANT AND ANIMAL FATS USING cDNA MICROARRAY

BY

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ABSTRACT

Halal and *haram* are ingrained in the daily life of a Muslim; guided by Al-Quran and As-Sunnah. This concerns of *halal* and *haram* has also opened a vast market operated by not only Muslims but non-Muslims all over the world. The rapid growth of *halal* market demands the use of technologies to ensure the quality and safety of *halal* products. These technologies range from compact and mobile test kits to the high-end techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Polymerase Chain Reaction (PCR). In line with the *halal* market growth, more scientific research related to *halal* by using the above techniques has also been reported. However, microarray has received very little attention in *halal* research. Therefore, this study explores the use of cDNA microarray to investigate the effects of fat from *haram* sources on HaCaT keratinocyte human skin cells in comparison to *halal* fat sources at gene expression level. The *haram* fat sources used in this study were lard and non-*halal* slaughtered lamb fat while Halal fat sources were virgin coconut oil (VCO) and *halal* slaughtered lamb fat. The RNAs extracted from treated cells were used in cDNA microarray (Agilent 8x60K SurePrint G3 Human GE). The data analyzed by GeneSpring GX 13.0, detected 50,739 genes from the four treatments and after further filtration; 53 genes were obtained with p-value of <0.05 and fold change of ≥ 2.0 (FC range between -2.457 to 6.813). The most regulated genes were NLRP5, FABP3, RPS21, PRKDC, ERCC4, ACTG1P4, and RACGAP1P. Selected genes (FABP3, PRKDC, GULP1, and XPOT) were then validated using real-time PCR. The gene expressions from real-time PCR were found to be consistent with microarray data. Finally, pathway analysis using Ingenuity Pathway Analysis (IPA® 2016) software gave some insights into the underlying molecular networks and pathways. Although the results were not entirely conclusive, some patterns were observed; the four fat emulsion treatments were involved in similar bio functionalities (cellular growth and proliferation, cell cycle and cellular movement) and associated diseases (developmental disorders involving the cell growth, connective tissue and hematological disease). In conclusion, the study showed that *halal* and *haram* fat sources caused differential gene expression in human cells. However, more work is warranted to further elucidate the pathways involved in order to understand the potential benefits and/or the perceived harmful effects of the fats.

خلاصة البحث

الحلال والحرم متأصلان في الحياة اليومية للمسلمين، منقادة بالقرآن الكريم والسنة. وقد فتح القلق من الحلال والحرم أيضا سوقاً واسعة ليس فقط من قبل المسلمين ولكن غير المسلمين في جميع أنحاء العالم. ويتطلب النمو السريع في سوق الحلال استخدام التكنولوجيا لضمان جودة وسلامة المنتجات الحلال. وتتراوح هذه التكنولوجيا بين مجموعات الاختبار المدمجة والمتنقلة إلى التقنيات المتطورة مثل التحليل الطيفي للأشعة تحت الحمراء (FTIR)، والمسح التفاضلي للمسح الكهربي (DSC)، وتفاعل البوليميراز المتسلسل (PCR). وتماشياً مع نمو السوق الحلال، تم أيضا القيام عن المزيد من البحوث العلمية المتعلقة بالحلال باستخدام التقنيات المذكورة أعلاه. ومع ذلك، تلقى ميكروأري القليل جدا من الاهتمام في بحوث الحلال. لذلك، تستكشف هذه الدراسة استخدام cDNA ميكروأري للتحقق من تأثير الدهون من المصادر المحرمة على هاكات الخلايا الكيراتينية خلايا الجلد البشرية HaCaT بالمقارنة مع مصادر الدهون الحلال في مستوى التعبير الجيني. وكانت مصادر الدهون المحرمة المستخدمة في هذه الدراسة هي دهن الخنزير ودهن الضأن المذبوح بطرق غير شرعية، بينما كانت مصادر الدهون الحلال زيت جوز الهند البكر (VCO) ودهن الضأن الحلال. تم استخدام الحمض النووي الريبي RNAs المستخرج من الخلايا المعالجة في cDNA ميكروأري (Agilent 8x60K SurePrint G3 Human GE). البيانات التي تم تحليلها كشفت عن 50،739 جيناً من العلاجات الأربعة وبعد مزيد من الترشيح. تم الحصول على 53 جيناً مع قيمة p <0.05 ومضاعف تغيير ≤2.0 (FC تتراوح بين -2.457 إلى 6.813). وكانت الجينات الأكثر تنظيماً هي NLRP5، FABP3، RPS21، PRKDC ، ERCC4، ACTG1P4، و RACGAP1P. تم التحقق من صحة الجينات المحددة (FABP3، PRKDC ، GULP1، XPOT) باستخدام الوقت الحقيقي PCR. تم العثور على أنّ التعبيرات الجينية من RT-PCR متسقة مع بيانات ميكروأري. وأخيرا، أعطى تحليل المسار باستخدام تحليل مسار الإبداع (IPA® 2016) بعض الرؤية في الشبكات الجزيئية الكامنة والمسارات. على الرغم من أن النتائج لم تكن قاطعة تماماً، لوحظت بعض الأنماط؛ حيث تشارك مستحلب الدهون الأربعة في وظائف حيوية مماثلة (النمو الخلوي والانتشار، ودورة الخلية والحركة الخلوية) والأمراض المرتبطة بها (اضطرابات النمو التي تنطوي على نمو الخلايا والنسيج الضام وأمراض الدم). وكاستنتاج، أظهرت الدراسة أن مصادر الدهون الحلال والحرام تسببت في التعبير الجيني التفاضلي في الخلايا البشرية. ومع ذلك، فإنه لابد من إجراء المزيد من الأبحاث لزيادة توضيح المسارات التي ينطوي عليها فهم الفوائد المحتملة أو الآثار الضارة المتصورة للدهون.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Halal Industry Science).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Halal Industry Science).

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DECLARATION

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LIST OF ABBREVIATIONS

ACTB	Beta-actin
ANOVA	Analysis of variance
cDNA	Complementary deoxyribonucleic acid
CO_2	Carbon dioxide
cRNA	Complementary ribonucleic acid
DHA	Docohexanoic acid
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
EPA	Eicosapentaenoic acid
EtBr	Ethidium bromide
EVOO	Extra virgin olive oil
FA	Fatty acid
FABP3	Fatty acid binding protein 3
FBS	Fetal bovine serum
FC	Fold change
FE	Feature extraction
FTIR	Fourier Transform Infrared
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GULP1	GULP, Engulfment adaptor PTB domain containing 1
HaCaT	Human keratinocyte skin cell
IPA	Ingenuity Pathway Analysis
mRNA	Messenger ribonucleic acid
PBS	Phosphate buffer saline
P.B.U.H	Peace be upon him
PCR	Polymerase chain reaction
PRKDC	Protein kinase, DNA-activated, catalytic polypeptide
qPCR	Quantitative polymerase chain reaction
R.A	*Radiallahu anhu*

RIN	RNA integrity number
RNA	Ribonucleic acid
RT-PCR	Real-Time polymerase chain reaction
S.W.T	*Subhanallahu wata'ala*
VCO	Virgin coconut oil
XPOT	Exportin tRNA

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Halal is an important concern and continuously revolves around Muslims and increasingly among other population as well. Worldwide, *halal* labels or logo are synonym to the assurance for the safety and wholesomeness of the products. *Halal* is comprehensive and does not cover only food but everything in daily basis such as cosmetics, personal care, pharmaceuticals, clothes, logistics, finance and trades. Muslims need to be cautious in determining what to consume and what not, and this is guided by the verse in the Quran:

> *"O mankind, eat from whatever is on earth [that is] lawful and good and do not follow the footsteps of Satan. Indeed, he is to you a clear enemy. "*
>
> *(Surah Al-Baqarah; 2: 168)*

In the present time, the increase of *halal* awareness helps to expand and flourish the *halal* market all around the world. To aid the understanding and *muamalah* of *halal* needs in human life, government and non-governmental organizations authorities are established. Interest in *halal* science also showed an increasing trend. For instance, there are studies on the effects of slaughtering on animals according to *halal* and *shechita* methods (Gregory et al., 2008) and the validation of food authentication by using high

end equipment such as Fourier transform infrared (FTIR) and polymerase chain reaction (PCR) (Rohman et al., 2011; Aida et al., 2005).

There are numerous experiments regarding *halal* issues can be elegantly performed and simplified throughout sophisticated technologies. To the best of our knowledge, the application of microarray technology in *halal* science related research is very limited. This high end technology has shown excellent performance in various fields of research such as cancer, toxicology, environmental safety, ecology, clinical genotyping and pathological testing (Brennan et al., 2005; Chin & Kong, 2002; Call et al., 2003; Huyghe et al., 2009). A study conducted by Liu and coworkers (2006) on gene expression of superior frontal cortex from alcoholic and non-alcoholic individuals reported that the alteration of genes from alcoholic person led to several neural diseases such as Alzheimer and other psychiatric diseases. This is the evidence to the prohibition of alcoholic beverages as Allah S.W.T mentioned in the Quran:

> *"They question you about strong drink (khamr) and games of chance. Say; in both is great abuse and usefulness for mankind; but the abusive side of them is greater than their usefulness. "*
>
> *(Surah Al-Baqarah; 2: 219)*

To this end, microarray technology especially cDNA microarray has a huge potential to be one of the useful tools of *halal* science research. In this present study, we used cDNA microarray to investigate the effect of *halal* and *haram* fats on human keratinocyte cells at the gene expression level.

1.2 PROBLEM STATEMENT

With the increasing of *halal* and *haram* awareness and market demands, a lot of work has been directed towards *halal* authentication of food and consumer products with major concerns on alcohol and components of pig origins. Various approaches and lab-based investigations such as enzymatic and proteomics have been applied in the *halal* science related research. However, less has been focused on the effect of *haram* substances at the gene expression level. In particular, little is known about the effects of swine components (the fats) on human at gene expression level despite the perceived harmful effects of pigs due to its *haram* status which is parallel to one of the Islamic principles on *halal* and *haram* stated by Al-Qhardawi (1994); prohibition of things due to their impurity and harmfulness. Components from swine are vastly being used in many areas such as baking, clothing and even constructions. It is therefore, the interest of this study to investigate the effects of fat from pig at the gene expression level using microarray analysis in comparison to other fat/lipid emulsions.

1.3 RESEARCH HYPOTHESIS

The different types of fat/lipid emulsions used to treat HaCaT human keratinocytes may result in different effects on cell behavior. The lipid emulsion from plant source which is from virgin coconut oil (VCO) may benefit the growth of cells as have been shown in nutraceuticals and cosmeceuticals. Meanwhile, the animal source of fat emulsion particularly lard, *halal* slaughtered lamb fat and non-*halal* slaughtered lamb fat may either encourage or inhibit cell growth.

The phenotypic characteristics of the cells, including the cell behavior upon treatment of the fat/lipid emulsions are the consequence of events occurring at the molecular level. Thus, investigating the effects at the gene expression may provide further depth of understanding of the underlying mechanisms. It is noteworthy that, this study is undertaken not to challenge the *shariah* prohibition of *haram* components in food and consumer products; rather it is to attest their harmful or negative effects. Microarray technology, which can simultaneously analyze thousands of genes, holds a great potential as a tool to decipher the effects of the different types of fats (*halal* and *haram*) on human cells and provide biomarker(s) that can be used to develop *halal* authentication protocols.

1.4 RESEARCH OBJECTIVES

The research objectives of this study are:

1. To investigate the effects of fat from *haram* sources on human skin keratinocyte (HaCaT) cells in comparison to *halal* fat sources at the gene expression level by microarray analysis.
2. To validate the result of microarray on *halal* and *haram* sources on HaCaT cells by using real-time PCR.
3. To identify the potential pathways related to the genes affected by the *halal* and *haram* fat emulsions on HaCaT cells.

1.5 RESEARCH METHODOLOGY

The major steps that were involved in this study are displayed in Figure 1.1. The detailed methodology is described in Chapter three of this thesis.

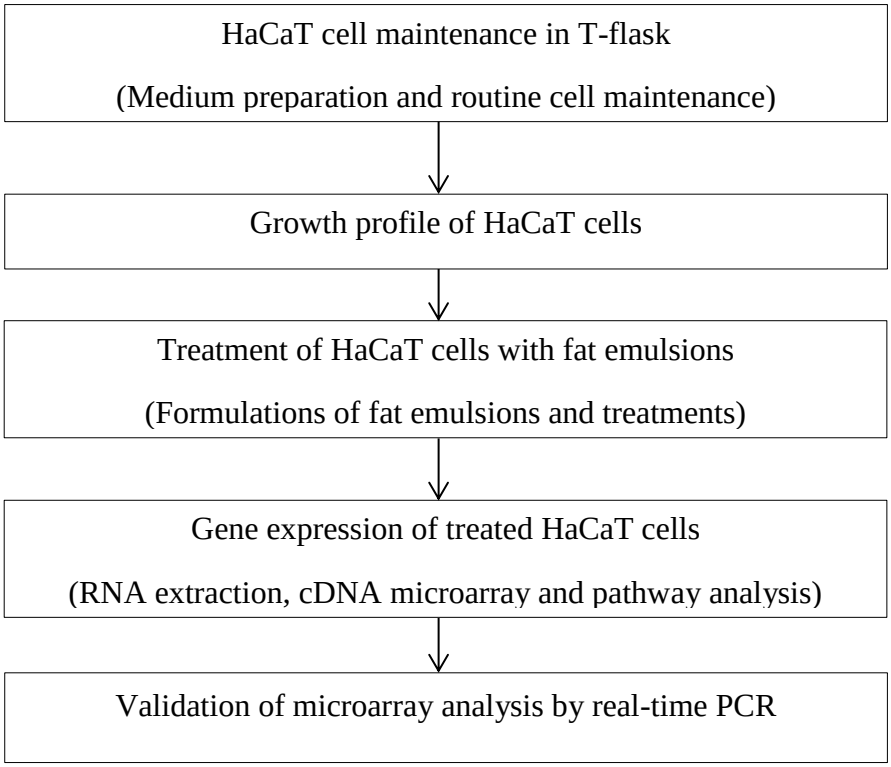

Figure 1.1: Application of cDNA microarray for *haram* detection of fat on HaCaT keratinocyte cells.

1.6 SCOPE OF STUDY

This study used HaCaT human keratinocyte cells as *in vitro* model to investigate the effects of different types of *halal* and *haram* fat sources on human cells at gene expression level. The fats used in the study were from virgin coconut oil (VCO), *shariah*

slaughtered lamb, conventional slaughtered lamb, and lard. These fats were incorporated in gum arabic solution to produce emulsions and used in the treatment of HaCaT cells. The cDNA microarray platform was used to study the gene expression from the treated cells as compared to non-treated cells. Real-time PCR was used to validate a selection of selected genes obtained from microarray work. Pathway analysis was carried out to provide insights of potential underlying mechanism of how the different fats affected the cells.

1.7 THESIS ORGANIZATION

The outline of the chapters in this thesis is as follows:

i. Chapter One comprises of background, problem statement, research hypothesis, objectives of the research, research methodology and scope of study.

ii. Chapter Two reveals the literature review that is related to the study on *halal* and *haram* and the gene expression study.

iii. Chapter Three discusses the materials and details of methodologies used in this study.

iv. Chapter Four presents the results and discussion on the experiments conducted.

v. Chapter Five gives the conclusions of the research with recommendations for further related studies.

CHAPTER TWO

LITERATURE REVIEW

In this chapter, the *halal* and *haram* definitions are described with details on the *halal* authorities and entities, *halal* science research and technologies involved with *halal* authentication as well as slaughtering in Islam. Meanwhile, the gene expression section is further detailed with subtopics of DNA microarray and its applications; and end with real-time PCR as validation technique for microarray.

2.1 *HALAL* AND *HARAM*

Halal and *haram* are pertinent in Islam and comprehensively covers daily life items including food, beverages, clothes, personal care products and cosmetics as well as services such as logistics, tourism and hospitality. Allah S.W.T. said in the Quran:

> *"O ye who believe! Eat of the good things wherewith We have provided you, and render thanks to Allah of it is (indeed) He whom ye worship."*
>
> *(Surah Al-Baqarah 2: 172)*

Allah has ordered Muslims to be concerned of what surrounds them. When *halal* sources are obtained, praises are due to Him the God Almighty. And when *halal* sources are very scarce, Muslims are urged to initiate and develop alternatives to the *haram* when