UNIVERSITI SAINS MALAYSIA
PROJEK PENYELIDIKAN JANGKA PENDEK
LAPORAN AKHIR

STUDY ON THE EFFECT OF BLACK CUMIN OIL AND LDL ON LIPOPOLYSACCHARIDE (LPS) – INDUCED TOLL – LIKE RECEPTOR ACTIVATION IN MACROPHAGE AND DENDRITIC CELL LINES

PENYELIDIK

DR. SHAHRUL BARIYAH SAHUL HAMID

PENYELIDIK BERSAMA

PROF. MYRON SZEWCZUK

Sedutan Minit Mesyuarat JKP-39 (07.Julai.2010)

3.	**Dr. Shahrul Bariyah Sahul Hamid** **(IPPT)** Study of the Effect of Black Cumin Oil and LDL On Lipopolysaccharide (LPS)- Induced TOLL-Like Receptor 4 Activation in Macrophage and Dendritic Cell Lines **(Laporan Akhir)**	Laporan Akhir Geran USM Jangka Pendek kajian ini diperakukan.

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. **Nama Ketua Penyelidik:** DR SHAHRUL BARIYAH SAHUL HAMID
Name of Research Leader

☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):**
School/Department

PUSAT PENGAJIAN SAINS PERUBATAN

3. **Nama Penyelidik Bersama:**
Name of Co-Researcher

PROFESSOR MYRON SZEWCZUK

4. **Tajuk Projek:**
Title of Project

Study on the Effect of Black Cumin Oil and LDL on Lipopolysaccharide (LPS)-Induced TOLL-like Receptor 4 Activation in Macrophage and Dendritic Cell Lines

5. **Ringkasan Penilaian/*Summary of Assessment:***	Tidak Mencukupi *Inadequate* 1	2	Boleh Diterima *Acceptable* 3	Sangat Baik *Very Good* 4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☑	☐	☐
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☑	☐	☐
iii) **Kualiti impak:** *Quality of impacts*	☑	☐	☐	☐	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☐	☑	☐	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☑	☐	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☑	☐	☐

6. **Abstrak Penyelidikan**
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**. Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar):

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Sila lihat lampiran

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Sila lihat lampiran

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
Receptor "Toll-like" 4	Toll-like Receptor 4
Minyak Jintan Hitam	Black Cumin Oil
Lipoprotein densiti rendah	Low density lipoprotein

8. **Output dan Faedah Projek**
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals

Manuskrip akan dihantar untuk penerbitan setelah satu lagi eksperimen dijalankan oleh pelajar M.Med.

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

Kajian perlu diteruskan secara komprehensif diperingkat pre-klinikal dan klinikal sebelum perkembangan produk.

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

Latihan Pelajar Sarjana

i) Pelajar Sarjana
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Dr Mahaya Che Mat
Sarjana Perubatan (M.Med Chemical Pathology)

Pelajar telah melakukan kerja makmal untuk memenuhi sebahagian dari objektif kajian ini. Satu lagi eksperimen pengekspresan receptor akan dijalankan untuk melengkapkan keseluruhan kajian sebelum manuskrip diserah untuk penerbitan.

ii) Lain-lain:
Others

9. Peralatan yang Telah Dibeli:
Equipment that has been purchased

Tiada

3 Mei 2010

Tandatangan Penyelidik
Signature of Researcher

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

The project has been completed and objectives have been achieved. The project has produced a M.Med clinical pathology and a manuscript of "Nigella sativa oil modulates oxidized low density lipoprotein uptake in primary human macrophages" has been submitted to Clinica Chimica Acta.

The report has been assessed by an independent assessor and approved by the PTJ research committee.

19/7/10

Tarikh
Date

BORANG LAPORAN HASIL PENYELIDIKAN
PPSP

Tajuk geran:Study of the Effect of Black Cumin Oil and LDL on Lipopolysaccharide (LPS)-induced TOLL-like Receptor 4 Activation in Macrophage and Dendritic Cell Lines
Penyelidik: Dr Shahrul Bariyah Sahul Hamid

Jenis laporan: Laporan Kemajuan (setiap 6 bulan) ☐ Alatan di beli ☐ Ya : nyatakan.....

Laporan Akhir*: ☑ ☑ Tidak

OBJEKTIF SPESIFIK KAJIAN (sama spt dalam proposal asal)	SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL	SEBAB-SEBAB JIKA TAK TERCAPAI
1. To grow primary monocyte, macrophage and dendritic cell lines	The primary macrophages were grown successfully using cell culture techniques in suitable growth media	Only macrophages cell lines were used in this study due to time limitation.
2. To study the chemical compositions of black cumin oil	We selected only 2 active ingredients thymoquinone and ρ-cymene which are mainly studied. The results showed black cumin oil and ρ-cymene have similar inhibitory function on TLR-4 activation. However, thymoquinone was found to activate the receptor.	Only two active ingredients were selected for further investigations. The entire chemical composition has been studied by other group of researchers.
3. To investigate the effect of black cumin oil on NFκB activation and LDL uptake using immunological and microscopic technique	Further investigation using immunological and microscopic method showed thymoquinone acts as an inhibitor on activation of NFκB. The final part of the study was conducted at PPSP. Primary monocytes were isolated from human whole blood using Dynabead isolation method. The cells were grown with oxLDL and treated with black cumin as described in methods in manuscript. Cell staining and microscopic techniques were utilized to observe the effects on cell growth from monocytes to macrophages. The presence of the oil was found slow the growth of macrophages. The final experiment on CD11b expression will be conducted to support this finding.	

** Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan.*

Nama Penyelidik Utama (PI): Dr Shahrul Bariyah Sahul Hamid t.t.:
Tarikh: 22 April 2010

Study on anti-lipidaemic effect of *Nigella sativa* oil on oxidized low density lipoprotein uptake by primary human macrophages

Mahaya CM[1], Tan Koh Chun[2], Wan Zuraida[3] Mohd Azman S[4], Shahrul BSH[1]

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[2] Central Research Laboratory, School of Medical Sciences, USM

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[4]School of Dental Sciences, USM

ABSTRACT

Lipid-laden macrophage has been reported to play various roles in atherogenesis. The focus was to elucidate effects of a natural product on the progression of monocyte-derived macrophage growth. *Nigella sativa* was selected as a form of treatment to macrophage cell growth in culture conditions due to various findings on its medical benefits. Human monocytes were isolated and grown at 37°C and 5%CO_2 saturation for 5 days prior to treatment with *Nigella sativa* oil. The cells were plated and washed before addition of ox-LDL (10μg/ml) alone in untreated condition and combination of ox-LDL (10μg/ml) and (72 μg/ml) *Nigella sativa* oil in treated condition. The growth progression was monitored every 24 hours for 3 days. *Nigella sativa* oil caused noticeble effect on macrophage growth compared to monocyte especially 24 hours after treatment. The mean was significant different between untreated and treated condition for both monocytes and macrophages ($p<0.001$). This was shown by the delayed growth pattern as seen in macrophage compared to monocytes. There was less oil red O staining in cells treated with mixture of oxLDL and *Nigella sativa* compared to those treated with oxLDL alone. The signalling of *Nigella sativa* has been reported to occur via TLR-4 receptor, where it is significantly present on macrophage cell surface. This may be one of the factors leading to the growth difference. Hence, progression of macrophage to foam cells could possibly be controlled with the use of *Nigella sativa* oil.

Introduction

Macrophages are key players in many aspects of human physiology and disease [1]. A hallmark of the development of atherosclerotic plaques is the prior and concurrent accumulation in the arterial intima of lipoprotein particles subject to chemical modifications. This is associated with local inflammation in the vessel wall and further recruitment of monocytes from the circulation. By taking up such modified LDL (oxidized or acetylated), monocyte-derived macrophages are turned into fat-loaded macrophages residing in the vessel wall and furthering the local inflammatory response. The mechanisms underlying such foam cell generation has for several years been the focus of intensive research [2,3,4,5,6]. The NF-B pathway is one of the main signaling pathways activated in response to proinflammatory cytokines, including TNF-, IL-1, and IL-18, as well as following activation of the Toll-like receptors (TLR) by the pat[illegible] recognition of pathogen-associated molecular patterns (PAMPs). Activ[illegible] pathway plays a central role in inflammation through the regulation of [illegible]