



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT, CHARACTERIZATION, AND INVESTIGATION OF
ANTI-DIABETIC PROPERTIES OF THYMOQUINONE RICH FRACTION
NANOEMULSION OBTAINED FROM BLACK CUMIN***

ZAKI A. H. TUBESHA

FPSK(p) 2013 16



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By

ZAKI A. H. TUBESHA

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

August 2013

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Chairman: Professor. Maznah Ismail, PhD

Faculty: Medicine and Health Sciences

For thousands of years, natural products have played important roles throughout the world in preventing and treating human diseases. Black cumin (*Nigella sativa L.*) and its bioactive constituents, thymoquinone (TQ), have been used for various health benefits such as antidiabetic, antitumor, antihypertensive, antioxidative and antibacterial agents. Type 2 diabetes mellitus (T2DM) which accounts for over 90 % of diabetes cases is currently a major chronic disease affecting about 6 % of the global population. Therefore, discovering new therapies is vital for management of T2DM. Although TQ and thymoquinone rich fraction (TQRF) are known to be powerful antioxidants and have hypoglycemic properties, their administration *in vivo* remains problematic partly due to their low bioavailability. Lipid nanoemulsions from some medicinal plant oils such as TQRF or from hydrophobic bioactives such as TQ are attractive candidates for improving solubility and bioavailability of the

oils and their bioactives. Therefore, the aim of the present study was to develop, characterize, and investigate antidiabetic properties of TQRF nanoemulsion (TQRFNE) and TQ nanoemulsion (TQNE). Conventional emulsions from TQRF (TQRFCE) and from TQ (TQCE) were also prepared for comparison. TQRF extracted from *N. sativa* using supercritical fluid extraction (SFE) contained 4.45 % TQ and was used to develop TQRFNE and TQRFCE. To develop TQNE and TQCE, Triolein (TR) was used as a carrier of commercially acquired TQ based on the concentration of TQ in TQRF. Conventional emulsions were prepared first by mixing 5 % lipid phase (TQRF or TR which contains calculated amount of TQ) with 95 % aqueous phase (2 % Tween-80, and 93 % distilled water) at room temperature using Ultra-Turrax mixer (13000 rpm / 3 min). Nanoemulsions were produced by passing the conventional emulsions through high pressure Homogenizer (800 bars and five homogenization cycles).

In the characterization study, the changes in particle size, zeta potential, polydispersity index, refractive index, and chemical stability (loss of TQ during storage) of all emulsions were investigated over a period of 6 months at three different storage temperatures (4, 25, and 40 °C). At the end of characterization period, all nanoemulsions displayed good physical and chemical stability; there was no phase separation or any sign of instability of all nanoemulsion samples. In the antioxidant study, all emulsion samples were tested for antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS^{•+}), and β -carotene bleaching (BCB) assays using UV- visible spectrophotometer, and hydroxyl radical scavenging activity by electron spin resonance (ESR) spectrometer. Antioxidant activities of all nanoemulsions were

slightly lower compared to conventional emulsions. In addition, acute toxicity of TQRFNE, and TQNE was studied using Sprague–Dawley rats. All the parameters measured were unaffected as compared to the negative control group (administered with distilled water). Further study was done to investigate the antidiabetic properties of the emulsions using T2DM animal model. Rats treated with TQRFNE and TQNE (10 ml/kg bw) exhibited a significant ($P < 0.05$) reduction in plasma total cholesterol levels (43 and 58 %), triglyceride (45 and 59 %), and fasting plasma glucose (42.1 and 54.9 %), respectively, compared to diabetic rats (DC group). However, TQRFCE and TQCE (10 ml/kg bw) also exhibited a significant ($P < 0.05$) reduction in plasma total cholesterol levels (56 and 69 %), triglyceride (57 and 67 %), and fasting plasma glucose (58 and 54.2 %), respectively, compared to DC group. For the liver and kidney function tests, the concentration of Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Urea (URE), and Creatinine (CREA) were also measured to test any toxic effect on all tested groups. All tested parameters were also significantly lower compared to DC group. For the gene expression study, genes related to glucose metabolism (FBP1), cholesterol metabolism (LDL-R) and antioxidants (SOD1) were evaluated in this study. Treatment with TQRFNE showed antihyperglycemic, hypocholesterolemic and antioxidant effects, partly through regulation of above mentioned genes. Taken together, our finding showed that TQRFNE as a novel nanocarrier has the potential to produce better metabolic outcomes in T2DM. In addition, the findings also suggest that TQRFNE may be better than metformin in management of T2DM, and that it potentially could improve the wellbeing of diabetics through better control of metabolic indices, and likely fewer side effects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN, PENCIRIAN DAN PENGAJIAN SIFAT- SIFAT ANTI-DIABETIK NANOEMULSI FRAKSI KAYA TIMOKUINON DARIPADA JINTAN HITAM

Oleh

ZAKI A. H. TUBESHA

Ogos 2013

Pengerusi: Professor. Maznah Ismail, PhD

Fakulti: Perubatan dan Sains Kesihatan

Sejak beribu tahun lamanya, hasil semulajadi telah memainkan peranan yang penting dalam mencegah dan merawat penyakit manusia di seluruh dunia. *Nigella sativa* dan sebatian aktifnya, timokuinon (TQ) telah digunakan untuk pelbagai tujuan kesihatan seperti antidiabetik, antitumor, antihipertensi, antioksidatif dan ejen antibakteria. Diabetes mellitus jenis 2 (T2DM) yang merangkumi lebih daripada 90% kes diabetik merupakan suatu penyakit kronik utama yang menjejaskan kira-kira 6% populasi global. Justeru, penemuan terapi baru adalah penting untuk pengurusan T2DM. Walaupun TQ dan fraksi kaya timokuinon (TQRF) sudah dikenalpasti sebagai antioksidan dan mempunyai sifat-sifat hipoglisemik, penyerapannya secara *in vivo* sebahagiannya masih bermasalah disebabkan oleh bioavailabilitinya yang rendah. Nanoemulsi lipid daripada sesetengah minyak tumbuhan ubatan seperti TQRF atau daripada bioaktif hidrofobik seperti TQ merupakan calon yang berpotensi untuk meningkatkan proses pelarutan dan bioavailabiliti minyak tersebut dan sebatian bioaktifnya. Justeru, kajian ini bertujuan untuk membangunkan, mencirikan dan mengkaji sifat-sifat antidiabetik nanoemulsi

TQRF (TQRFNE) dan nanoemulsi TQ (TQNE). Nanoemulsi konvensional daripada TQRF (TQRFCE) dan daripada TQ (TQCE) juga disediakan untuk perbandingan. TQRF yang diekstrak daripada minyak *N. sativa* dengan menggunakan pengekstrakan bendalir superkritikal (SFE) mengandungi 4.45% TQ dan digunakan untuk menghasilkan TQRFNE dan TQRFCE. Untuk menghasilkan TQNE dan TQCE, Triolein (TR) digunakan sebagai pembawa TQ yang diperolehi secara komersil berdasarkan kandungan TQ dalam TQRF. Emulsi konvensional disediakan dahulu dengan mencampurkan 5% fasa lipid (TQRF atau TR yang mengandungi jumlah TQ yang telah dikenalpasti) dengan 95% fasa larutan (2% Tween-80, dan 93% air suling) pada suhu bilik dengan menggunakan Ultra-Turrax mixer (13 000 rpm / 3 min). Nanoemulsi dihasilkan dengan melakukan emulsi konvensional melalui Homogenizer bertekanan tinggi (800 bar dan lima kitar homogenisasi). Dalam kajian pencirian, perubahan saiz partikel, keupayaan zeta, indeks polydispersiti, indeks refraktif, dan kestabilan kimia (kehilangan TQ semasa proses penyimpanan) bagi semua emulsi telah dikaji dalam jangka masa 6 bulan pada tiga suhu penyimpanan yang berbeza (4, 25 dan 40°C). Pada peringkat akhir tempoh pencirian, semua nanoemulsi menunjukkan keadaan fizikal dan kestabilan kimia yang baik; tiada pemisahan fasa atau tanda-tanda ketidakstabilan bagi semua sampel nanoemulsi tersebut. Dalam kajian antioksidan, semua sampel emulsi diuji untuk aktiviti antioksidan menggunakan diphenyl-2-picrylhydrazyl (DPPH[•]), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS^{•+}), dan ujian pelunturan β -carotene (BCB) dengan menggunakan spektrofotometer UV-boleh dilihat, dan aktiviti memerangkap radikal hidroksil menggunakan spectrometer resonan pemusingan electron (ESR). Aktiviti antioksidan semua nanoemulsi adalah sedikit rendah berbanding emulsi konvensional. Tambahan lagi, kesan toksik akut TQRFNE dan TQNE dikaji ke atas tikus Sprague-Dawley. Emulsi tersebut diberi secara oral kepada tikus bagi kedua-dua jantina pada hari 0 dan dikaji selama 14 hari. Kesan nanoemulsi ke atas berat badan,

pengambilan makanan dan minuman, berat relatif organ, hematologi, dan biokimia klinikal telah ditentukan. Kesemua parameter yang diambil tidak menunjukkan sebarang perubahan berbanding kumpulan kawalan negatif (diberikan air suling). Kajian lanjut telah dijalankan untuk mengkaji sifat antidiabetik emulsi tersebut dengan menggunakan model haiwan diabetis jenis 2. Tikus yang diberi TQRFNE dan TQNE (10 ml/kg bw) masing-masing menunjukkan penurunan yang signifikan bagi paras jumlah kolesterol dalam plasma (43 dan 58%), trigliserida (45 dan 59%) dan paras glukosa dalam plasma (42.1 dan 54.9%) berbanding tikus diabetik (kumpulan DC). Walaubagaimanapun, TQRFCE dan TQCE (10 ml/kg berat badan) masing-masing juga menunjukkan penurunan yang signifikan ($p < 0.05$) bagi paras kolesterol dalam plasma (56 dan 69%), trigliserida (57 dan 67%), dan paras gula dalam plasma (58 dan 54.2%) berbanding kumpulan DC. Untuk ujian fungsi hati dan buah pinggang, kepekatan Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Urea (URE) dan kreatinin (CREA) juga diukur bagi menguji kesan toksik ke atas semua kumpulan yang diuji untuk mengkaji sebarang kesan toksik ke atas kesemua kumpulan. Kesemua parameter yang diuji menunjukkan penurunan yang signifikan berbanding kumpulan DC. Untuk kajian kawalatur gen, gen-gen berkaitan metabolisme glukosa (FBP1), metabolisme kolesterol (LDL-R) dan antioksidan (SOD1) telah dikaji dalam eksperimen ini. Rawatan dengan TQRFNE menunjukkan sifat antihiperlipisemik, hipokolesterolemik dan kesan antioksidan, sebahagiannya menerusi pengawalan gen-gen tersebut. Kesimpulannya, keputusan kajian ini menunjukkan TQRFNE sebagai pembawa-nano yang novel adalah berpotensi untuk mencetus hasil metabolik yang lebih baik dalam T2DM. Tambahan pula, keputusan kajian ini mencadangkan bahawa TQRFNE mungkin lebih baik daripada metformin dalam pengurusan T2DM, dan dapat menambahbaik kesejahteraan pesakit diabetik melalui kawalan indeks metabolik yang lebih baik, dan kesan sampingan yang kurang.

ACKNOWLEDGEMENTS

I would like to express my utmost and deepest gratitude and appreciation to my wonderful supervisor Prof. Dr. Maznah Ismail for her towering presence, continuous effort, and guidance. Her valuable experience was of tremendous help and was always a source of ideas. Thanks would also be extended to my co-supervisors, Prof. Dr. Zuki Abu Bakar and Prof. Dr. Rozi Mahmud for their valuable comments.

Many thanks also goes to everybody in the staff of Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, for their kind and considerate cooperation, providing a friendly atmosphere around the lab. Special thanks go to my father, mother and siblings, thank you for tirelessly giving of support and encouragement. Your prayers, support and encouragement gave me the motivation to further pursue my education and fulfill my dreams. My sincere thanks and appreciation to my father-in-law for his support and editing this work.

I also take this opportunity to express my gratitude and appreciation to my loving wife Wafaa and to my three precious and beautiful girls Rama, Mais and Bana who offered me unconditional love and support throughout the course of this thesis.

Last but definitely not least, one special thanks goes to Islamic development bank for giving me scholarship. It would not have been possible to accomplish this work without their support. I find it inevitable to express my deepest appreciation for all what they have done for me.

I certify that a Thesis Examination Committee has met on 29th September / 2013 to conduct the final examination of Zaki AH. Tubesha on his thesis entitled "Development, Characterization, and Investigation of Anti-Diabetic Properties of Thymoquinone Rich Fraction Nanoemulsion Obtained from Black Cumin" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mahiran Basri, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Muhajir Hamid, PhD

Professor
Faculty of Biotechnology and
Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Asma Yahaya, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Hamed Takruri, PhD

Professor
Faculty of Agriculture
University of Jordan
(External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2013

This thesis submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as followed:

Maznah Ismail, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Zuki Abu Bakar, PhD

Professor
Faculty of veterinary medicine
Universiti Putra Malaysia
(Member)

Rozi Mahmud, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



ZAKI A. H. TUBESHA

Date:

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