UNIVERSITI PUTRA MALAYSIA

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AIDA ZARFESHANI

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AIDA ZARFESHANI

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To everyone who believed my abilities,

And supported me in my intention,

To make some of my dreams come true.

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

EFFECTS OF *LACTOBACILLUS CASEI* CONSUMPTION ON INFLAMMATION BIOMARKERS AND HISTOLOGICAL CHANGES IN SELECTED ORGANS IN NORMAL AND DIABETIC RATS

By

AIDA ZARFESHANI

November 2010

Chairman: Assoc. Prof. Mohd Sokhini Bin Abd Mutalib, PhD

Faculty: Medicine and Health Sciences

The severity of diabetes mellitus often manifested by a progressive inflammation, indicated by increased in circulating inflammatory biomarkers. Reducing the rate of the inflammation progression is one of the many measures to reduce complication of the disease. Many established evidences have suggested the beneficial effect of probiotic consumption on the progression of inflammatory bowel syndrome (IBS). In the present study, possible benefit of probiotics on inflammatory progression of Diabetes mellitus (DM) is investigated. The present study employed two different approaches to induce hyperglycemia in adult *Sprague- Dawley* rats. The initial approach using high fructose diet (HFD), (21% w/v), was unable to induce satisfactorily hyperglycemia in the animal. Chemical induction using streptozotocin (STZ), (50 mg/ kg body weight) induced hyperglycemia in all animals injected. Rats in both batches were divided into four groups. A non-diabetic group (ND), a non-treated group with a standard diet (NT) and two diabetic groups which were treated with 10^9 cfu/ml/day (LC1/DLC1) and 10^{11} cfu/ml/day

(LC2/DLC2) of self-cultured *Lactobacillus casei* strain *Shirota (LcS).* The probiotic *L.cS* was *gavaged* for three consecutive weeks. Blood was collected through the orbital venous plexus to measure circulating C-reactive protein (CRP), interleukin-6 (IL-6) and interleukin-4 (IL-4) as the inflammatory biomarkers. The results have shown no significant difference in blood glucose level of *L.cS* fed rats compared with non-treated group ($p>0.05$). Both doses of *L.cS* were observed to induce lower CRP production after three weeks of administration compared to the diabetic control group. Interleukin-6 was found to be decreased but only at higher dose (10^{11} cfu/ml) of the *L.cS* which was comparable with the level that was observed in the non-diabetic group at the end of the study duration. Interleukin-4 level was found to be significantly decreased in all treated and the diabetic control group, but was observed to be higher in the normal group. Data from the three inflammatory biomarkers suggested that probiotic *L.cS* has the potential to improve inflammatory status among STZ-induced rats. Histological study of the pancreas showed a huge damage of the Langerhans islets in STZ induced rats. In addition, foamy cells were found in the kidney cortex of the STZ induced rats. Colon condition was similar among all groups. Inflammatory invasion was evidenced in the liver where the presence of neutrophils could be clearly seen in the non-treated group. Treatment with the *L.cS* observed a significant reduction in the invasiveness of the neutrophils which highly indicates decreased levels of inflammation by the probiotics. The results of this study indicate that *Lactobacillus casei* strain *Shirota*, possess an ability to reduce inflammatory biomarkers hence complication caused by inflammation in DM.

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

KESAN PENGAMBILAN *LACTOBACILLUS CASEI* PERHADAP PENANDA BIOLOGI KERADANGAN DAN PERUBAHAN HISTOLOGI DALAM ORGAN TERPILIH BAGI TIKUS NORMAL DAN DIABETES

Oleh

AIDA ZARFESHANI

November 2010

Pengerusi : Professor Madya Mohd Sokhini Bin Abd Mutalib, PhD

Fakulti : Perubatan dan Sains Kesihatan

Tingkat keparahan diabetes mellitus (DM) sering dimanifestasikan oleh keradangan progresif, ditunjukkan dalam peningkatan edaran penanda biologi radang. Mengurangkan kadar keradangan adalah salah satu langkah untuk mengurangkan komplikasi penyakit ini. Banyak bukti menunjukkan kesan pengambilan probiotik adalah bermanfaat untuk sindrom radang usus (IBS). Kajian ini melaporkan potensi probiotik dalam keradangan progresif DM. Oleh itu, dua pendekatan berbeza digunakan dalam kajian ini untuk mengaruhkan hiperglisemia pada tikus dewasa Sprague-Dawley. Pendekatan awal adalah dengan menggunakan diet fruktosa tinggi yang didapati tidak memuaskan dalam mengaruhkan hiperglisemia tikus. Pengaruhan kimia menggunakan streptozotocin (STZ), (50 mg/ kg berat badan) dapat mengaruhkan hiperglisemia pada semua haiwan yang disuntik. Tikus dibahagikan kepada empat kumpulan iaitu satu kumpulan kawalan normal (ND), kumpulan kawalan diabetes dengan diet piawai (NT) dan dua kumpulan diabetes yang dirawat dengan 10^9 cfu/ml/hari (DLC1/LC1) dan 10^{11} cfu/ml/hari (DLC2/LC2) kultur *Lactobacillus casei* strain Shirota (*LcS*). Probiotik diberikan kepada tikus

selama tiga minggu berturut-turut secara oral atau *gavage*. Darah diambil diawal dan diakhir kajian melalui pleksus vena orbit dan seterusnya digunakan untuk mengukur aras edaran Interleukin-4 (IL-4), Interleukin-6 (IL-6) dan protein C-reaktif sebagai penanda biologi keradangan dan lain-lain parameter biologi. Keputusan kajian menunjukkan tiada perbezaan signifikan pada aras glukosa darah tikus yang diberi *L.cS* berbanding kumpulan kawalan diabetes ($p<0.05$). Kedua-dua dos *L.cS* mendorong pengeluaran CRP yang lebih rendah selepas tiga minggu rawatan berbanding dengan kumpulan kawalan diabetes. IL-6 menunjukkan penurunan tetapi hanya pada *L.cS* dos tinggi (DLC2/LC2, 10^{11} cfu/ml) yang seterusnya dapat dibandingkan dengan kumpulan ND diakhir kajian. Aras IL-4 dilihat menurun secara signifikan pada semua tikus yang dirawat dan kumpulan NT tetapi lebih tinggi pada kumpulan ND. Data daripada tiga penanda biologi keradangan menunjukkan probiotik *LcS* mempunyai potensi dalam memperbaiki status keradangan pada tikus aruhan STZ. Kajian histologi pankreas menunjukkan kerosakan serius pada kelompok Langerhans tikus aruhan STZ. Selain itu, sel berbusa ditemui di korteks ginjal tikus diabetes aruhan-STZ. Kolon berada pada tahap normal bagi tikus sihat dan lain-lain kumpulan diabetes. Kehadiran neutrofil dapat dilihat dengan jelas pada kumpulan NT yang membuktikan keradangan pada organ hati. Rawatan *L.cS* didapati dapat menurunkan tahap kemansangan neutrofil secara signifikan yang seterusnya menunjukkan penurunan radang disebabkan oleh *L.cS*. Keputusan kajian ini menunjukkan bahawa *Lactobacillus casei* strain *Shirota*, betpotensi untuk mengurangkan penanda biologi keradangan yang menyebabkan komplikasi DM.

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I certify that an Examination Committee has met on 16/Nov/2010 to conduct the final examination of Aida Zarfeshani on her Master of Sciences thesis entitled "Effects of *Lactobacillus Casei* consumption on levels on inflammation biomarkers and histological changes in selected organs in normal and diabetic rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the relevant degree.

Members of Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of sciences. The members of the Supervisory Committee were as follows:

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Date: 18 January 2011

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.

AIDA ZARFESHANI

Date:

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ABBREVIATIONS

ANOVA: Analysis Of Variance

ATP: Adenosine-5'-Tri Phosphate

BG: Blood Glucose

CFU: Colony Forming Unit

CRP: C-reactive Protein

DM: Diabetes Mellitus

DLC1: Diabetic *L.cS* 1

DLC2: Diabetic *L.cS* 2

ELISA: Enzyme Linked Immunosorbent Assay

FAO: Food and Agriculture Organization

GDM: Gestational Diabetes Mellitus

GLUT2: Glucose Transporter 2

GLUT4: Glucose Transporter 4

H and E stain: Hematoxylin and Eosin stain

HFD: High Fructose Diet

IBD: Inflammatory Bowel Disease

IBS: Irritable Bowel Syndrome

IDDM: Insulin Dependent Diabetes Mellitus

IFN-γ: Interferon- γ

IκBα: Inhibitor Kappa B Alpha

IκBβ: Inhibitor Kappa B Beta

IL-1,1 b,4,6,10: Interleukin-1,1 b,4,6,10

ILs: Interleukins

LC1: *L.cS* 1

LC2: *L.cS* 2

L.cS: *Lactobacillus casei* strain *Shirota*

MANOVA: Multivariate Analysis of Variance

MNT: Medical Nutrition Therapy

MRS: de Man, Rogosa and Sharpe

NAD: Nicotinamide Adenine Dinucleotide

ND: Non-diabetic

NT: Non-treated

NFκB: Nuclear Factor Kappa B

NIDDM: Non Insulin Dependent Diabetes Mellitus

NK: Natural Killer

NO: Nitric Oxide

PCR: Polymeras Chain Reaction

ROS: Reactive Oxygen Species

RPM: Revolutions Per Minutes

STZ: Streptozotocin

TG: Triglyceride

TNF-α: Tumor Necrosis Factor Alpha

WHO: World Health Organization

Weight/Volume: w/v

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