UNIVERSITI PUTRA MALAYSIA

ANTIDIABETIC AND IMMUNOLOGICAL EFFECTS OF GINGER RHIZOME ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

MANSOOREH SADAT MOJANI QOMI

FPSK(p) 2014 15

ANTIDIABETIC AND IMMUNOLOGICAL EFFECTS OF GINGER RHIZOME ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

By

MANSOOREH SADAT MOJANI QOMI

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

December 2013

DEDICATION

This thesis is dedicated to my loving parents who have supported me all the way of my life.
Also, this thesis is dedicated to my beloved husband who has been a great source of motivation and inspiration.
Finally, this work is dedicated to my little lovely angel, tasnim, with a hope that she enjoys her life with health, happiness and success.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

Antidiabetic and Immunological Effects of Ginger Rhizome on Streptozotocin-Nicotinamide Induced Diabetic Rats

By

MANSOOREH SADAT MOJANI QOMI

December 2013

Chairman: **Asmah Rahmat, PhD**

Faculty: **Medicine and Health Sciences**

The present study was done to determine anti-diabetic and immunological effects of ginger rhizome (*Zingiberis officinale*) in healthy and nicotinamide-streptozotocin (NA-STZ) induced-diabetic rats. The Characteristics of ginger rhizome were investigated by determination of macronutrients composition using proximate analysis, determination of active components using HPLC method, determination of flavonoid content using aluminium chloride calorimetric assay, total phenolic compound using Folin-Ciocaltea reagent and anti-oxidant activity using DPPH radical scavenging assay. The results showed that young Malaysian ginger which was used for the treatment of rats had high amount of moisture content, less carbohydrate and energy contents compared with the ginger from other regions in previous studies. It had the highest level of 6-gingerol. Total flavonoid and phenolic content was 3.66±0.45 mg quercetin and 10.22±0.87 mg gallic acid per gram of dry weight of rhizome, and DPPH radical scavenging activity was 51.4±0.4% of free radical inhibition.

The study was undertaken to determine the effects of ginger rhizome on NA-STZ induced-diabetic and healthy rats. Male Sprague-dawley rats were injected a single intraperitoneal dose of nicotinamide prior to STZ. Following 72 hours of injection, those rats with blood glucose level more than 200 mg/dl (equivalent to 11.1 mmol/l) were selected as diabetic rats. A total of 72 rats

were divided into 9 groups (4 normal groups and 5 diabetic-induced groups); three different dosages of ginger rhizome were examined (250, 500 and 750 mg/kg body weight). Finally, the results were compared with the control groups.

In animal experiments, independent samples t-test showed statistically significant changes in terms of fasting blood glucose, body weight, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL-c) and tumor necrosis factor-α (TNF-α) between diabetic and healthy rats following diabetic induction (p<0.05). Low density lipoprotein (LDL-c), interleukin-6 (IL-6), and C-reactive protein (CRP) remained unchanged. Following 6-weeks intervention, lymphocyte proliferation was impaired in response to the lowest concentration of mitogen (1 μg/ml), and T cells in both unstimulated (p=0.001) and stimulated states (p=0.031 for LPS and p=0.001 for PHA) significantly failed to response. Once the levels of stimuli increased to 5 μg/ml, the cells showed more activations, but still the decline was noted in diabetic rats (p>0.05). Data from phenotyping assay also demonstrated that the only difference was seen in the percentage of $CD4^+CD25^+$ cell numbers (a marker of regulatory T cells) that was higher in diabetic rats (p<0.05).

The effects of ginger rhizome on treated group were later analyzed using one-way ANOVA followed by LSD post hoc test. The results indicated that intervention had some inhibitory effects on weight gain; most ginger-treated groups had lower body weight compared with their controls (p<0.01). This finding was well supported by the rats' food intake. Both blood fasting and plasma glucose of rats were lower in dosages of 250 and 500 mg/kg of diabetic groups compared with the control rats (p<0.05). Total cholesterol and triglyceride did not change following treatment except diabetic rats treated with 250 mg/kg in which the level of triglyceride decreased significantly (p<0.05), LDL-c and HDL-c were significantly decreased in the diabetic group of 500 mg/kg B.W. Decreasing HDL-c led to an increase of the atherogenic indexes in a dose-dependent manner.

Supplementation of ginger rhizome showed no effects on the level of CRP in diabetic rats. Levels of IL-6 did not change; nevertheless, levels of TNF-α significantly decreased in all the diabetic-treated rats. The efficiency of treatment was evident by changes in *p value* consistent with increasing dosage of ginger rhizome.

In proliferation assay, PHA stimulation with 1 μg/ml caused a significant activation in normal group with 250 mg/kg body weight and diabetic groups with two lower ginger concentrations (250 and 500 mg/kg B.W.). Moreover, PHA stimulation with 5 μg/ml produced a considerable proliferation in all the

treated groups including glibenclamide (p<0.05); in contrast, stimulation with LPS did not affect any treated groups in two experiments. The results of phenotyping assay reported no significant changes in number of T helper cells ($CD3^+CD4^+$), $CD4^+$ alone, percentage of T cells and Natural killer cells, but markers of regulatory T cells ($CD4^+CD25^+$) were significantly raised in normal and diabetic rats with dosages of 750 and 500 respectively (p<0.05). The percentage of B cells also significantly increased in the lowest dosage of ginger in both normal and diabetic rats.

To sum up, the results clearly showed that ginger rhizome supplementation in lower dosages regulated blood glucose in diabetic condition; also it showed benefits on lowering levels of TG, LDL-c, TNF-α and some markers of immune functions. Although the advantages of ginger are evident from the findings of this study and previous literature, further research is recommended to be done in human subjects to confirm the current results.

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

Kesan Antidiabetik Dan Immunologikal Rizom Halia Di Dalam Tikus Diabetik Yang Diaruh Streptozotocin-Nicotinamide

Oleh

MANSOOREH SADAT MOJANI QOMI

Disember 2013

Pengerusi: Asmah Rahmat, PhD

Fakulti: Perubatan dan Sains Kesihatan

Kajian ini telah dilaksanakan untuk menentukan kesan anti-diabetik dan immunologikal oleh rizom halia (*Zingiber isofficinale*) bagi tikus sihat dan diaruh diabetik dengan *nicotinamide streptozotocin* (NA-STZ). Ciri-ciri rizom halia telah disiasat dengan penentuan komposisi makronutrien dengan menggunakan analisis proksimat, penentuan komponen-komponen aktif menggunakan kaedah kromatografi cecair berprestasi tinggi (HPLC), penentuan jumlah kandungan fenolik menggunakan reagen Folin-Ciocalteu dan aktiviti anti-oksidan menggunakan kaedah penghapusan radikal bebas 2,2-diphenyl-1-picrylhydrazyl (DPPH). Keputusan telah menunjukkan halia muda dari Malaysia yang digunakan untuk rawatan bagi tikus mempunyai kandungan air yang tinggi, karbohidrat dan tenaga yang rendah berbanding halia dari kawasan lain yang ditemui dalam kajian lepas. Ia mempunyai paras 6-gingerol yang tinggi. Jumlah flavonoid dan fenolik ialah masing-masing 3.66±0.45 mg quercetin dan 10.22±0.87 mg asid galik per gram berat kering rizom, serta aktiviti penghapusan radikal DPPH ialah 51.4±0.4% perencatan radikal bebas.

Kajian telah dijalankan untuk menentukan kesan rizom halia ke atas tikus diaruh NA-STZ dan tikus sihat. Tikus jantan Sprague-dawley telah disuntik satu dos nikotinamid secara *intraperitoneal* sebelum STZ. Selepas 72 jam penyuntikan, tikus yang didapati dengan paras glukosa dalam darah lebih daripada 200 mg/dl (bersamaan dengan 11.1 mmol/l) telah terpilih sebagai tikus diabetik. Sejumlah 72 ekor tikus telah dibahagikan kepada 9 kumpulan

(4 kumpulan normal dan 5 kumpulan tikus diaruh diabetik); tiga dos rizom halia yang berbeza telah diuji (250, 500 dan 750 mg/kg berat badan). Akhir sekali, keputusan telah dibandingkan dengan kumpulan kawalan.

Dalam eksperimen haiwan, ujian t sampel tidak bersandar telah menunjukkan perubahan yang signifikan bagi glukosa darah berpuasa, berat badan, trigeliserida (TG), jumlah kolesterol (TC), lipoprotein berketumpatan tinggi (HDL-c) dan faktor-α nekrosis tumor (TNF-α) di antara tikus sihat dan diabetik berikutan aruhan diabetik (p<0.05). Lipoprotein berketumpatan rendah (LDL-c), interleukin-6 (IL-6) dan protein C-reaktif telah kekal tidak berubah. Selepas 6 minggu intervensi, tindakan proliferasi limfosit telah terjejas kepada kepekatan mitogen terendah (1 μg/ml), dan sel T bagi kedua-dua keadaan iaitu tidak dirangsang (p=0.001) dan rangsang (p=0.031 untuk LPS dan p=0.001 untuk PHA) telah gagal untuk bertindak secara signifikan. Apabila paras perangsang ditingkatkan kepada 5 μg/ml, sel tersebut telah menunjukkan pengaktifan lebih tinggi, tetapi penurunan masih ditemui dalam tikus diabetik (p>0.05). Data dari kaedah fenotip juga mempamerkan perbezaan hanya bagi peratus bilangan sel $CD4^+CD25^+$ (penunjuk sel aturan T) di mana ia lebih tinggi dalam tikus diabetik (p<0.05).

Kesan rizom halia ke atas kumpulan rawatan telah dianalisa menggunakan *one-way* ANOVA diikuti ujian LSD. Keputusan telah menunjukkan intervensi tersebut memberi kesan perencatan ke atas pertambahan berat; kebanyakan kumpulan rawatan dengan halia mempunyai berat badan lebih rendah berbanding kumpulan kawalan (p<0.01). Penemuan ini telah disokong dengan pengambilan makanan oleh tikus. Kedua-dua puasa darah dan glukosa plasma tikus rendah untuk dos 250 dan 500 mg/kg B.W. bagi kumpulan diabetik (p<0.05). Jumlah kolesterol dan trigeliserida tidak berubah dengan rawatan kecuali tikus diabetik dengan dos 250 mg/kg, di mana paras trigeliserida menurun secara signfikan (p<0.05), LDL-c and HDL-c juga menurun secara signifikan dalam kumpulan diabetik dengan dos 500 mg/kg. Penurunan HDL-c telah membawa kepada peningkatan indeks atherogenik dengan bergantung pada dos.

Suplimentasi rizom halia telah menunjukkan tiada kesan ke atas paras CRP dalam tikus diabetik. Kandungan IL-6 tidak berubah; namun begitu kandungan TNF-α dalam semua tikus diabetik yang dirawat telah berkurang secara signifikan. Keberkesanan rawatan telah dibuktikan oleh perubahan nilai P yang konsisten dengan pertambahan dos rizom halia.

Dalam kaedah proliferasi, stimulasi PHA dengan 1 μg/ml telah menyebabkan pengaktifan yang signifikan dalam kumpulan normal dengan 250 mg/kg dan kumpulan diabetik dengan dua kepekatan halia yang rendah (250 dan 500 mg/kg). Selain itu, stimulasi PHA dengan 5 μg/ml menghasilkan satu proliferasi besar dalam semua kumpulan rawatan termasuklah *glibenclamide* (p<0.05); disebaliknya stimulasi LPS tidak mempengaruhi mana-mana

kumpulan rawatan dalam dua eksperimen. Keputusan dari kaedah fenotip telah melaporkan tiada perubahan yang signifikan dalam bilangan sel pembantu T ($CD3^+CD4^+$), $CD4^+$, peratus sel T dan sel *Natural killer,* tetapi penunjuk sel pengawalan T ($CD4^+CD25^+$) telah menaik secara signifikan ($p<0.05$) bagi tikus sihat dan diabetik, masing-masing dengan dos 750 dan 500 mg/kg. Peratus sel B juga telah meningkat secara signifikan dengan dos halia yang paling rendah bagi kedua-dua kumpulan tikus sihat dan diabetik.

Secara keseluruhan, keputusan jelas menunjukkan suplimentasi rizom halia dengan dos rendah telah mengawal gula dalam darah bagi keadaan diabetik; ia juga menunjukkan kelebihan dengan penurunan paras TG, LDL-c, TNF-α dan beberapa penunjuk fungsi imun. Walaupun kelebihan halia adalah bukti dari penemuan dalam kajian ini dan kajian lepas, penyelidikan lanjut di masa hadapan ke atas subjek dicadangkan untuk mengesahkan keputusan terkini.

ACKNOWLEDGEMENTS

Apart from the efforts of me, the success of any project depends largely on the encouragement and guidelines of many others. I take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this work. I would like to express the deepest appreciation to my committee chair person, Professor Dr. Asmah Rahmat, who has the attitude and the substance of a genius: She continually and convincingly conveyed a spirit of adventure in regard to research and an excitement in regard to teaching. Without her guidance and persistent help completing this thesis would not have been possible.

I also would like to thank my committee members, Dr. Rajesh Ramasamy and Dr. Loh Su Peng for their valuable remarks, meticulous reading and invaluable comments at all stages of this research. I would also like to extend my special thanks to my postgraduate friends Vahid Hosseinpour Sarmadi, Pratheep Sandrasaigaran, Noridzzaida Ridzuan and Dr. Shalini Vellasamy for their kindly help in immunology lab affairs in some parts of proliferation and phenotyping assays. My sincere thanks go to the supporting staff of animal house, Nutrition, Pathology and Immunology laboratories, faculty of Medicine and Health Sciences, UPM.

I would like to thank my loved ones: my parents for their faith, encouragement, love and understanding. Finally, this thesis is dedicated to my husband, Dr. Seyed Majid Akhavan Hejazi, and my beloved daughter, Tasnim, for their never ending supports.

I clarify that a Thesis Examination Committee has met on 13/12/2013 to conduct the final examination of Mansooreh Sadat Mojani Qomi on her thesis entitled “Antidiabetic and Immunological Effects of Ginger Rhizome on Streptozotocin-Nicotinamide Induced Diabetic Rats” 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:

Mohd Sokhini Abd Mutalib, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Fauziah binti Othman, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Patimah binti Ismail, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Lindsay Brown, PhD
Professor
Faculty of School of Health, Nursing and Midwifery
University of Southern Queensland
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 21 January 2014

This thesis was submitted to the Senate of 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 follows:

Asmah Rahmat, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Loh Su Peng, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Rajesh Ramasamy, PhD
Associate 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: 13 February 2014

DECLARATION

I declare that the dissertation is my original work except for the quotation 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.

MANSOOREH SADAT MOJANI QOMI

Date:13December2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ABCC8	ATP-binding cassette transporter sub-family C member 8
Abs	Absorbance
ACTH	Adrenocorticotropic Hormone
ACN	Acentonitrile
ACUC	Animal Care and Use Committee
ADA	American Diabetic Association
ADP	Adenosine Diphosphate
$AlCl_3$	Aluminium chloride
ANOVA	Analysis Of Variance between Groups
AP-1	Activator Protein-1
APC	Allophycocyanin
ATP	Adenosine triphosphate
BHT	Butylated hydroxytoluene
BMI	Body Mass Index
BSA	Bovine Serum Albumin
BW	Body Weight
C	Carbon
CALPN10	calpain 10
CD	Cluster of Differentiation
Con A	Concanavalin A
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CPM	Count per Minute
CRP	C-reactive Protein
CVD	Cardio Vascular Disease
db/db mice	A model of obesity, diabetes, and dyslipidemia
$^{\circ}C$	Degree Centigrade
12-DHGB	12-Dehydrogingerdione
dl	Deciliter
DPPH	Diphenylpicryl- hydrazyl
DQ	αβ heterodimer of the MHC Class II type
DTH	Delayed Type Hypersensivity
Egr-1	Early Growth Response-1
ELISA	Enzyme-linked immunosorbent assay
FCS	Fetal Calf Serum
FCR	Immunoglobulin Receptor
FITC	Fluorescein isothiocynate
FSC	Forward Scattered
eq.	Equivalent
Foxp3	Forkhead box P3
g	Gram
G	Glibenclamide

GDM	Gestational Diabetes Mellitus
Glut-4	Glucose transporter type 4
HDL-c	High Density Lipoprotein Cholesterol
HF	High Fat
HLA-DP	α,β Hetero-diomer Cell Surface Receptor
HLA-DR	Human Leukocyte Antigen-DR
HPLC	High-performance Liquid Chromatography
IC_{50}	half Maximal Inhibitory Concentration
ICAM-1	Intracellular Adhesion Molecules-1
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IFN-γ	Interferon-γ
IκB	Inhibitor Kappa-B
IL-6	Interluekin-6
IP	Intraperitoneal
KCNJ11	Potassium Inwardly-rectifying Channel, Subfamily J, Member 11
Kg	Kilogram
L	Litre
LDL-c	Low Density Lipoprotein Cholesterol
LPS	Lipopolysaccharide
LSD	Least Significant Difference
m^2	Square Meter
MAPK	P38 Mitogen Activated Protein Kinase
MCP-1	Monocyte Chemo-attractant protein-1
MeOH	Methanol
MLC	Mixed Lymphocyte Culture
mg	Milligram
μg	Microgram
μl	Microliter
mmol	Milli mol
MHC class	Major Histocompatibility Complex Class
mM	Milli mol
MMP-9	Metallo proteinase-9
MNCs	Mononulear cells
mRNA	Messenger Ribonucleic acid
MSG	Mono Sodium Glutamate
NA	Nicotinamide
NAD^+	Aldehyde Dehydrogenase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
$NaNO_2$	Sodium Nitrite
NEFAs	Non-sterified Fatty Acids
NFκB	Nuclear Factor kappa-B
NK cells	Natural Killer cells
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
P47phox	47-kilodalton Cytosolic Subunit of the Multi-protein

	Complex
PAI-1	Plasminogen activator inhibitor-1
PE	Phycoerythrin
PE-cy5	PE-cyanin-5
%	Percent
PerCP	Peridin-chlorophyll Protein
PG-E2	Prostaglandin E2
pg	Picogram
pH	Measure of the activity of the (solvated) hydrogen ion
PHA	Phytohaemagglutinin
PMA	Phorbol Myristate Acetate
PPARγ	Peroxisome Proliferator-activated Receptors
PTFE Filter	Polytetrafluoroethylene Filter
RBC	Red Blood Cell
RBL-1 cell	Rat Hematopoietic Leukemia cell line
ROS	Reactive Oxygen Species
rpm	Revolutions per Minute
RPMI	Roswell Park Memorial Institute (A Cell Culture Media)
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SEM	Standard Error of Mean
SSC	Side-Scattered
STZ	Streptozotocin
TC	Total Cholesterol
TCR	T cell Receptor
T2D	Type 2 Diabetes
TG	Triglyceride
TNF-α	Tumor Necrosis Factor-alpha
TPC	Total Phenolic Content
Treg	Regulatory T cells
TXB2	Tromboxane B-2
USP	US Pharmacopeial Convention
UV	Ultra Violet
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Background

Ginger is the rhizome of the plant *Zingiber officinale Roscoe* which is consumed as a delicacy or spice. The name originates from its genus and family (Zingiberaceae). It was first cultivated in India and Southeast Asia, and then introduced to other regions of the world. This traditional medicine has been used among the Chinese, Indian and Japanese for more than 25 centuries (Castleman, 2001). Ginger is also used in a variety of diseases, particularly gastrointestinal disorders like constipation, diarrhea, anorexia, colic, dyspepsia, nausea, vomiting and morning sickness. Pungent principles of ginger are gingerols; they are biologically active components that may make a significant contribution towards medicinal applications of ginger (Sanwal *et al*., 2010).

Diabetes Mellitus is a chronic disease which is mostly recognized in two main forms: type 1 or insulin-dependent diabetes in which pancreatic β-cells are gradually damaged and there is no secretion or little secretion of insulin. Type 2 or non-insulin-dependent diabetes is a heterogeneous disorder in which despite presence of insulin, there is insulin resistance and pancreatic β-cell dysfunction (2010).

It is hypothesized that chronic subclinical inflammation not only is associated with insulin resistance (Thorand *et al*., 2005), but also has a role in the pathogenesis (Spranger *et al.,* 2003) and development of clinically evident type 2 diabetes (Thorand *et al*., 2005). It was shown that the pattern of circulating inflammatory cytokines such as Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) synthesis due to IL-6 stimulation modifies the risk for type 2 diabetes (Spranger *et al*., 2003). In type 2 diabetic patients, leukocyte cell count was raised as well as higher expression of activation markers of neutrophils and monocytes (van Oostrom *et al*. 2004).

Problem Statement

Diabetes mellitus is a chronic disease that requires long-term medical attention both to limit the development of its overwhelming complications and

to manage them when they do occur. It is a disproportionately expensive disease; in 2002, the per-capita cost of health care was US$13,243 for people with diabetes compared with $2560 for non-diabetic diseases (Laditka, *et al*., 2001). Rates of diabetes are increasing worldwide. At least 171 million people currently have diabetes, and this figure is likely to more than double to 366 million by 2030 (WHO, 2011). In addition, another 41 million people are estimated to have pre-diabetes, which includes impaired glucose tolerance (IGT) (2-hour post-challenge glucose of 140-190 mg/dl) and impaired fasting glucose (IFG) (fasting plasma glucose 100-125 mg/dl) (Centers for Disease control and prevention, 2005). People with pre-diabetes are at high risk for conversion to type 2 diabetes and cardiovascular disease (CVD). Elevating markers of inflammation in pre-diabetes conditions change this process much faster; on the other hand, increasing levels of inflammatory cytokines cause many troubles for diabetic patients: who experience atherosclerosis due to inflammatory factors and development of microvascular diabetic complications including nephropathy (Navarro and Mora-Ferna´ndez, 2006) and diabetic retinopathy (Joussen *et al*., 2004).

Totally, Diabetes mellitus contributes to a considerable increase in morbidity and mortality rates, which can be reduced by early diagnosis and treatment. Additionally, high rates of diabetes impose many costs on inpatient care, outpatient services and nursing home care that can be reduced if life style prevention strategies are implemented.

Significance of Study

The current public health nutrition guideline announced some anti-inflammatory strategies to tackle type 2 diabetes: to achieve and maintain a healthy weight, to lessen saturated fat, to increase the proportion of less refined forms of carbohydrate and to increase intake of fruits and vegetables (Browning and Jebb, 2006). Although today much of the evidence regarding the effects of nutrients and foods on disease has been based on epidemiological associations, human dietary intervention trials and studies on animals are required to discover links among diet, inflammation and diabetes. Some nutraceuticals such as α-tocopherol, ascorbic acid, curcumin, theaflavin, genistein, omega-3 fatty acid and lycopene are well-known to have anti-inflammatory properties. Some studies highlighted anti-inflammatory effects of ginger in rodent models (Habib *et al*., 2008; Fatehi-

Hassanabad, 2005, Thomson *et al.,* 2002), but did not clarify this role in diabetes and hyperglycemia. The present study tried to be different in aspects of anti-inflammatory effect of ginger which mainly occurs in diabetic state and to investigate the function of ginger, lipid profile measured in streptozotocin-nicotinamide induced diabetic rats. To elucidate more anti-diabetic and immunological effects of ginger rhizome, the current study looks at the proliferation of lymphocytes (spleen T cells), the effect of ginger on lymphocyte immunophenotyping, as well as, some characteristics of ginger rhizome by using the relevant methods.

Research Objectives

General Objective

To study anti-diabetic and immunological effects of ginger rhizome (*Zingiber officinale Roscoe*) in streptozotocin-nicotinamide induced diabetic rats

Specific Objectives

The specific objectives of this study are as followed:

1. To determine the constituents of ginger rhizome based on:
   i. The proximate composition;
   ii. The amounts of active components (6- , 8- , 10-gingerol and 6- , 8-, 10-shagaol),
   iii. Total flavonoids (TF)
   iv. Total phenolic content (TPC),
   v. Antioxidant activity,
2. To evaluate metabolic and immunological changes between healthy and streptozotocin-nicotinamide induced diabetic rats,
3. To investigate effects of ginger on body weight changes and food intake of the streptozotocin-nicotinamide induced diabetic rats,
4. To determine hypoglycemic effects of ginger in the streptozotocin-nicotinamide induced diabetic rats,
5. To determine hypocholesterolemic and hypolipidemic effects (total cholesterol, triglyceride , HDL-c and LDL-c) of ginger in the streptozotocin-nicotinamide induced diabetic rats,
6. To determine effects of ginger on cytokines (IL-6, TNF-α and CRP) in the streptozotocin-nicotinamide induced diabetic rats,

7. To determine effect of ginger on lymphocyte proliferation in the streptozotocin-nicotinamide induced diabetic rats,
8. To investigate immunophenotyping effect of ginger rhizome on lymphocyte subpopulations (helper T cells, regulatory T cells, natural killer T cell, B cells and T cells) in the streptozotocin-nicotinamide induced diabetic rats.

Hypothesis

H_A *1*: There is a relationship between ginger and body weight and food intake of the streptozotocin-nicotinamide induced diabetic rats

H_A *2:* Ginger rhizome regulates blood glucose levels in the streptozotocin-nicotinamide induced diabetic rats,

H_A *3:* Ginger normalizes levels of lipid profile in the streptozotocin-nicotinamide induced diabetic rats,

H_A*4:* Ginger decreases levels of inflammatory biomarkers (IL-6, TNF-α and CRP) in the streptozotocin-nicotinamide induced diabetic rats,

H_A *5:* Ginger improves lymphocyte proliferation in the streptozotocin-nicotinamide induced diabetic rats,

H_A *6:* Ginger improves lymphocyte subpopulations in the streptozotocin nicotinamide induced diabetic rats.

REFERENCES/ BIBLIOGRAPHY

Abdulrazaq, N. B., Cho, M. M., Win, N., Zaman, R., & Rahman, M. T. (2011). Beneficial effects of ginger (Zingiber officinale) on carbohydrate metabolism in streptozotocin-induced diabetic rats. *British Journal of Nutrition*, 1-8.

Afzal, M., Al-Hadidi, D., Menon, M., Pesek, J., & Dhami, M. S. (2001). Ginger: an ethnomedical, chemical and pharmacological review. . *Drug Metab. Drug Interact., 18*, 159-190.

Agnaniet, H., Menut, C., & Bessiere, J. M. (2004). Aromatic plants of tropical central Africa. Part XLIX: Chemical composition of essential oils of the leaf and rhizomes of Aframomum giganteumK. Schum from Gabon. . *Flavour and Fragrance Journal, 19*, 205-209.

Akhani, S. P., Vishwakarma, S. L., & Goyal, R. K., 101-105. (2004). Anti-diabetic activity of Zingiber officinale in streptozotocin-induced type I diabetic rats. *Journal of Pharmacy and Pharmacology, 56*(1), 101-105.

Al-Amin, Z. M., Thomson, M., Al-Qattan, K. K., Peltonen-Shalaby, R., & Ali, M. (2006). Anti-diabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin-induced diabetic rats. *British Journal of Nutrition,, 96*, 660–666.

Al-Awadi, F. M., & Gumaa, K. A. (1987). Studies on the activity of individual plants of an antidiabetic plant mixture. *Diabetologica, 24*, 37-41.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Helper T Cells and Lymphocyte Activation. In *Molecular Biology of the Cell* New York: Garland Science.

Al-Qattan, K., Thomson, M., & Ali, M. (2008). Garlic (Allium sativum) and ginger (Zingiber officinale) attenuate structural nephropathy progression in streptozotocin-induced diabetic rats. *The European e-Journal of Clinical Nutrition and Metabolism, 3*, 62-71.

Albright, A., Franz, M., Hornsby, G., Kriska, A., Marrero, D., Ullrich, I., et al. (2000). American College of Sports Medicine position stand. Exercise and type 2 diabetes. *Medicine and Science in Sports and Exercise, 32*(7), 1345-1360.

Alenzi, F. Q. (2009). Effect of Nicotinamide on Experimental Induced Diabetes. *Iranian Journal of Allergy, Asthma & Immunology , 8*(1), 11-18.

Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. *Food and Chemical Toxicology 46*(2), 409-420.

Aljada, A., Ghanim, H., Mohanty, P., Kapur, N., & Dandona, P. (2002). Insulin Inhibits the Pro Inflammatory Transcription Factor Early Growth Response Gene-1 (Egr)-1 Expression in Mononuclear Cells (MNC) and Reduces Plasma Tissue Factor (TF) and Plasminogen Activator Inhibitor-1 (PAI-1) Concentrations. *The Journal of Clinical Endocrinology and Metabolism 87*(3), 1419-1422.

Alnajjar, A., Chabane Sari, D., Abuharfeil, N., Hudaib, M., & Aburjai, T. (2006). Effect of n-3 and n-6 polyunsaturated fatty acids on lymphocyte proliferation, interleukin production and phospholipid fatty acids composition in type 2 diabetic and healthy subjects in Jordan people. *Prostaglandins, Leukotrienes and Essential Fatty Acids 74*, 347-356.

American Diabetes Association (2006). Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 29, S43-S48.

American Diabetes Association (2003). Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Follow-up report on the diagnosis of diabetes mellitus. *Diabetes* 26, 3160-3167.

American Diabetes Association (1998). Standards of medical care for patients with diabetes mellitus. *Diabetes Care* 21, 23-31.

American Diabetes Association (1997). Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20,1183-1197.

Andlauer, W., & Fürst, P. (1998). Antioxidative power of phytochemicals with special reference to cereals. *Cereal Food. World 43*, 356-360.

Anonymous. (1997). Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care 20*, 1183-1197.

Anosike , C. A., Obidoa, O., Ezeanyika, L. U. S., & Nwuba, M. S. (2009). Anti-inflammatory and anti-ulcerogenic activity of the ethanol extract of ginger (Zingiber officinale). *African journal of microbiology research 3*(12), 379-384.

AOAC. (1990). Official methods of analysis, 16th edition. Gaithersburg, Maryland, USA: AOAC International.

B.D.Biosciences. (2002). *Introduction to Flow Cytometry: A Learning Guide*. San Jose: Becton, Dickinson Biosciences.

Badreldin, H. A., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. *Food and Chemical Toxicology.* 46(2),409-420.

Barroso, I., Luan, J., Middelberg, R. P. S., Harding, A.-H., Franks, P. W., Jakes, R. W., et al. (2003). Candidate gene association study in type 2 diabetes indicates a rolefor genes involved in B-Cell function as well as insulin action. *PLOS Biology 1*, 41-55.

Bergholdt, R., Heding, P., Nielsen, K., Nolsøe, R., Sparre, T., Stør-ling, J., et al. (2004). Type 1 diabetes mellitus: an inflammatory disease of the islet. *Advances in Experimental Medicine and Biology 552*, 129-153.

Bever, B. O., & Zahnd, G. R. (1979). Plants with oral hypoglycemic action. *Quarterly journal of crude drug research, 17*, 139-146.

Bhandari, U., Sharma, J. N., & Zafari, R. (1998). The protective action of ethanolic ginger (Zingiber officinale) extract in cholesterol fed rabbits. *Journal of Ethnopharmacology, 61*, 167- 171.

Bhattarai, S., Tran, V. H., & Duke, C. C. (2001). The stability of gingerol and shogaol in aqueous solutions. *Journal of Pharmaceutical Sciences 90*, 1658-1664.

Bilous, R., & Donnelly, R. (2010). Epidemilogy and Aetiology of Type 2 Diabetes. In *Hanbook of Diabetes* (pp. 44-52): Blackwell Publishing Ltd.

Black, S. (1962). Regulation of sulfoxide and disulfides. In Kolowick & N. Kaplan (Eds.), *Methods in enzymology* (Vol. 5, pp. 992-996). New York: Academic Press.

Blaschek, W., Ha¨ nsel, R., Keller, L., Reichling, J., Rimpler, H., & Schneider, G. (1998). *Hagers Handbuch der Pharmazeutischen Praxis*. Berlin, Heidelberg, New York: Springer.

Boutayeb, A., Lamlili, M. E. N., & Boutayeb, W. (2012). Obesity: Modeling the effect on human biomass and the impact on type 2 Diabetes. *Diabetes & Metabolism, 38*, S89-S116.

Breemen, R. B. V., Tao, Y., & Li, W. (2011). Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale). *Fitoterapia 82*, 38-43.

Buchanan, T. A., Xiang, A. H., Peters, R. K., Kjos, S. L., Marroquin, A., Goico, J., et al. (2002). Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. *Diabetes 51*(9), 2796-2803.

Bhushan, M.S., Rao, C.H.V., Ojha, S.K., Vijayakumar, M., Verma, A. (2010). An analytical review of plants for anti diabetic activity with their phytoconstituent & mechanism of action. *International Journal of Pharmaceutical Sciences and Research* 1(1), 29-46.

Butt, M. S., & Sultan, M. T. (2011). Ginger and its Health Claims: Molecular Aspects. . *Critical Reviews in Food Science and Nutrition, 51*, 383-393.

Bystry, R. S., Aluvihare, V., Welch, K. A., Kallikourdis, M., & Betz, A. G. (2001). B cells and professional APCs recruit regulatory T cells via CCL4. *Nature Immunology* 2, 1126-1132.

Caballero, A. E. (2005). Metabolic and Vascular Abnormalities in Subjects at Risk for Type 2 Diabetes: The Early Start of a Dangerous Situation. *Archives of Medical Research, 36*(3), 241-249.

Calder, P. C. (2001). The immune system is a complex system involving various cells and lymphoid organs. *Lipids 36*, 1007–1024.

Calle, M. C., & Fernandez, M. L. (2012). Inflammation and type 2 diabetes. *Diabetes & Metabolism, 38*(3), 183-191.

Centers for Disease Control and Prevention: National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2005, Atlanta, Ga, U.S. Department of Health and Human Services.

Chakraborty, D., Mukherjee, A., Sikdar, S., Paul, A., Ghosh, S., & Khuda-Bukhsh, A. N. (2012). [6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice. *Toxicology Letter 210*, 34-43.

Chang, F.-Y., & Shaio, M.-F. (1995). Decreased cell-mediated immunity in patients with non-insulin-dependent diabetes mellitus. *Diabetes Research and Clinical Practic, 28*, 137-146.

Chen, C.-C., Rosen, R. T., & Ho, C.-T. (1986). Chromatographic analyses of isomeric shogaol compounds derived from isolated gingerol compounds of ginger (zingiber officinale roscoe). *Journal of Chromatography A 360*(C), 175-184.

Chrubasik, S., Pittler, M. S., & Roufogalis, B. D. (2005). Zingiberis rhizoma: A comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine 12*, 684-701.

Clegg, K. M. (1995). The application of the anthrone reagent to the estimation of starch in cereals. *Journal of the Science of Food and Agriculture,* 7(1), 40-44.

Copeland, S., Siddiqui, J., & Remick, D. (2004). Direct comparison of traditional ELISAs and membrane protein arrays for detection and quantification of human cytokines. *Journal of Immunological Methods 284*(1-2), 99-106.

Dandona, P., Aljada, A., Mohanty, P., Ghanim, H., Bandyopadhyay, A., & Chaudhuri, A. (2003). Insulin suppress plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. *Diabetes Care 26*(12), 3310-3314.

Dandona, P., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W., Assian, E., et al. (2001). Insulin inhibits intranuclear nuclear factor kappa-B and stimulates I-kappa-B in mononuclear cells in obese subjects: evidence for an anti-inflammatory effects? *The Journal of Clinical Endocrinology & Metabolism 86*(7), 3257-3265.

Davis, R. H., & Maro, N. P. (1989). Aloe veraand gibberellins, Anti-inflammatory activity in diabetes. *Journal of the American Podiatric Medical Association, 79*, 24-26.

Dean, L., & McEntyr, J. (2004). *The Genetic Landscape of Diabetes*. National Center for Biotechnology Information (US): NCBI.

Delves, P. J., & Roitt, I. M. (2000). The immune system. Part 1. *New England Journal of Medicine 343*, 3-49.

Donath, M. Y., Størling, J., Maedler, K., & Mandrup-Poulsen, T. (2003). Inflammatory mediators and islet β-cell failure: a link between type 1 and type 2 diabetes. *Journal of Molecular Medicine 81*, 455-470.

Dorman, J. S., & Bunker, C. H. (2000). HLA-DQ locus of the human leukocyte antigen complex and type 1 diabetes mellitus:a HuGE review. *Epidemiologic Reviews 22*, 218-227.

Eizirik, D. L., & Mandrup-Poulsen, T. (2001). A choice of death-the signal-transduction of immune-mediated beta-cell apoptosis. . *Diabetologia, 44*, 2115-2133.

Eizirik, D. L., Sandler, S., Welsh, N., Cetkovic-Cvrlje, M., Nieman, A., Geller, D. A., et al. (1994). Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. *Journal of Clinical Investigation 93*, 1968-1974.

Elbein, S. C. (2002). Perspective: the search for genes for type 2 diabetes in the post-genome era. *Endocrinology, 143*, 2012-2018.

Eldershaw, T. P., Colquhoun, E. Q., Dora, K. A., Peng, Z. C., & Clark, M. G. (1992). Pungent principles of ginger (Zingiber officinale) are thermogenic in the perfused rat hindlimb. *International journal of obesity and related metabolic disorders 16*(10), 755-763.

Elkhishin, I., & Awwad, I. A. (2009). A Study of the Cardiovascular Toxic Effect of Zingiber Officinale (Ginger) in Adult Male Albino Rats and Its Possible Mechanisms of Action. *Mansoura Journal of Medicine Department of Forensic Medicine and Clinical Toxicology, 17*(2), 109-125.

Evans, J. L., Goldfine, I. D., Maddux, B. A., & Grodsky, G. M. (2002). Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocrine Reviews 23*, 599-622.

Fatehi-Hassanabad, Z., Gholamnezhad, Z., Jafarzadeh, M., & Fatehi, M. (2005). The Anti-Inflammatory Effects of Aqueos Extract of Ginger Root in Diabetic Mice. *Daru, 13*(2), 70-73.

Feutren, G., Papoz, L., Assan, R., Vialettes, B., Karsenty, G., Vexiau, P., Rodier, M., Sirmai, J., Lallemand, A. (1986). Cyclo-sporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet 2*, 119-124.

Flynn, D. L., Rafferty, M. F., & Boctor, A. M. (1968). Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. *Prostaglandins, Leukotrienes and Medicine 24*, 195-198.

Franz, M. J. (2008). Medical Nutrition Therapy for Diabetes Mellitusand Hypoglycemia of Non-Diabetic Origion. In S. K. Mahan & S. Escott-Stump (Eds.), *Krauses Food and Nutrition Therapy* (12th ed., pp. 764-809): Saunders, Elsevier Inc.

Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy* 8(3), 1-6.

Geerlings, S. E., & Hoepelman, A. I. M. (1999). Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunology and Medical Microbiology 26*, 259-265.

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2010). Identification and Concentration of Some Flavonoid Components in Malaysian Young Ginger (Zingiber officinale Roscoe) Varieties by a High Performance Liquid Chromatography Method. *Molecules, 15*, 6231-6243.

Giannoukakis, N. (2006). Drug evaluation: ranirestat-an aldose reductase inhibitor for the potential treatment of diabetic complications. *Current opinion in investigational drugs, 7*, 916-923.

Giugliano, D., Ceriello, A., & Esposito, K. (2008). Glucose metabolism and hyperglycemia. *Ameican Journal of Clinical Nutrition, 87*, 217S-222S.

Gouni-Berthold, I., Betteridge, D. J., & Krone, W. (2007). Dyslipidemia and Diabetes. In B. J. Goldstein & D. Müller-Wieland (Eds.), *Type 2 Diabetes: Principles and Practice* (pp. 405-416): Informa Healthcare.

Goyal, R. K., & Kadnur, S. V. (2006). Beneficial effects of Zingiber officinale on goldthioglucose induced obesity. *Fitoterapia, 77*, 160-163.

Grzanna, R., Phan, P., Polotsky, A., Lindmark, L., & Frondoza, C. G. (2004). Ginger extract inhibits beta-amyloid peptide-induced cytokine and chemokine expression in cultured THP-1 monocytes. *Journal of Alternative and Complementary Medicine 10*, 1009-1013.

Ha, S. K., Moon, E., Ju, M. S., Kim, D. H., Ryu, J. H., Ohc, M. S., et al. (2012). 6-Shogaol, a ginger product, modulates neuroinflammation: A new approach to neuroprotection. *Neuropharmacology 63*, 211-223.

Habib, S. H. M., Makpol, S., Hamid, N. A., Das, S., Ngah, W. Z. W., & Yusofl, Y. A. M. (2008). Gingerextract (Zingiber officinale) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatomarats. *Clinics, 63*(6), 807-813.

Han, Y. A., Song, C. W., Koh, W. S., Yon, G. H., Kim, Y. S., Ryu, S. Y., et al. (2012). Anti-inflammatory Effects of the Zingiber officinaleroscoe Constituent 12-Dehydrogingerdione in Lipopolysaccharide-stimulated Raw 264.7 Cells. *Phytotherapy Research 27,*1200-1205.

Hansen, L. (2003). Candidate genes and late-onsettype 2 diabetes mellitus.Susceptibility genes or common polymorphisms? *Danish medical bulletin 50*, 320-346.

Hegazy, H. G. (2011). Ameliorative effects of ginger and -lipoic acid on oxidative stress and inflammation in senile female rats. *African Journal of Pharmacy and Pharmacology 5*(8), 1096-1105.

Heuser, G., & Vojdani, A. (1997). Enhancement of natural killer cell activity and T and B cell function by buffered vitamin C in patients exposed to toxic chemicals: the role of protein kinase-C. *I Immunopharmacology and Immunotoxicology 19*, 291-312.

Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., & Spiegelman, B. M. (1995). Increased adipose tissue ex-pression of tumor necrosis factor-a in human obesity and insulin resistance. *Journal of Clinical Investigation 95*, 2409-2415.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-a: direct role in obesity-linked insulin resistance. *Science 259*, 87-91.

Iranloye, B. O., Arikawe, A. P., Rotimi, G., & Sogbade, A. O. (2011). Anti-diabetic and anti-oxidant effects of Zingiber Officinale on alloxan-induced and insulin-resistant diabetic male rats. *Nigerian Journal of Physiological Sciences 26*(1), 89-96.

Isa, Y., Miyakawaa, Y., Yanagisawaa, M., Gotob, T., Kangb, M., Kawadab,T., Morimitsuc, Y. Kubotac, K. Tsuda, T. (2008). 6-Shogaol and 6-gingerol, the pun-gent of ginger, inhibit TNF-α mediated downregulation of adiponectin expression via different mechanisms in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications 373*, 429-443.

Islam, M. S., & Loots Du, T. (2009). Experimental rodent models of type 2 diabetes: a review. *Methods and Findings in Experimental and Clinical Pharmacology 31*(4), 249-261.

Islam, S., & Choi. (2008). Comparative Effects of Dietary Ginger (Zingiber officinale) and Garlic (Allium sativum) Investigated in a Type 2 Diabetes Model of Rats. . *Journal of Medicinal Food 11*(1), 152-159.

Jafri, S. A., S., A., & M., Q. (2010). Hypoglycemic Effect of Ginger (Zingiber officinale)in Alloxan Induced Diabetic Rats (Rattus norvagicus). *Pakistan Veterinary Journal 31*(2), 160-162.

Jagannathan-Bogdan, M., McDonnell, M. E., Shin, H., Rehman, Q., Hasturk, H., Apovian, C. M., et al. (2011). Elevated Proinflammatory Cytokine Production by a Skewed T Cell Compartment Requires Monocytes and Promotes Inflammation in Type 2 Diabetes. *The Journal of Immunology 186*, 1162-1172.

Jeschke, M. G., Klein, D., Bolder, U., & Einspanier, R. (2004). Insulin Attenuates the Systemic Inflammatory Response in Endotoxemic Rats. . *Endocrinology 145*(9), 4084–4093.

John, C. M., Ramasamy, R., Al Naqeeb, G., Al-Nuaimi, A. H. D., & Adam, A. (2012). Nicotinamide Supplementation Protects Gestational Diabetic Rats by Reducing Oxidative Stress and Enhancing Immune Responses. *Current Medicinal Chemistry 19*(30), 5181-5186.

Jolad, S. D., Lantz, R. C., Solyom, A. M., Chen, G. J., Bates, R. B., & Timmermann, B. N. (2004). Fresh organically grown ginger (Zingiber officinale): Composition and effects on LPS-induced PGE2 production. *Phytochemistry 65*(13), 1937-1954.

Jung, M., Park, M., Lee, H. C., Kang, Y. H., Kang, E. S., Kim, S. K. (2006). Anti-diabetic agents from medicinal plants. *Current Medicinal Chemistry* 13, 1203-1218.

Kadnur, S. V., & Goyal, R. K. (2005). Beneficial effects of Zingiber officinale Roscoe on fructose induced hyperlipidemia and hyperinsulinemia in rats. *Indian Journal of Experimental Biology 43*, 1161-1164.

Kasai, S., Shiku, H., Torisawa, Y., Nagamine, K., Yasukawa, T., Watanabe, T., Matsue, T. (2006). Cytokine assay on a cellular chip by combining collagen gel embedded culture with scanning electrochemical microscopy. *Analytica Chimica Acta 566*, 55–59.

Kawada, T., Sakabe, S., Watanabe, T., Yamamoto, M., & Iwai, K. (1988). Some pungent principles of spices cause the adrenal medulla to secrete catecholamine in anesthetized rats. *Proceedings of the Society for Experimental Biology and Medicine 188*, 229-233.

Kim, S. O., Kundu, J. K., Shin, Y. K., Park, J. H., Cho, M. H., Kim, T. Y., Surh, Y. J. (2005). [6]-Gingerol inhibits COX-2 expres-sion by blocking the activation of p38 MAP kinase and NF-κB in phorbol ester-stimulated mouse skin. *Oncogene, 24*, 2558-2567.

Kiuchi, F., Iwakami, S., Shibuya, M., Hanaoka, F., & Sankawa, U. (1992). Inhibition of prostaglandin and leukotriene biosynthesis by gingerols

and diarylheptanoids. *Chemical and Pharmaceutical Bulletin (Tokyo) 40*, 387-391.

Kiuchi, F., Shibuya, M., & Sankawa, U. (1982). Inhibitors of prostaglandin biosynthesis from ginger. *Chemical and Pharmaceutical Bulletin (Tokyo), 30*, 754-757.

Kruisbeek, A. M., Shevach, E., & Thornton, A. M. (2004). Proliferative Assays for T Cell Function. *Current Protocols in Immunology 3*(12), 3-20.

Krutzik, P. O., Clutter, M. R., & Nolan, G. P. (2005). Coordinate Analysis of Murine Immune Cell Surface Markers and Intracellular Phosphoproteins by Flow Cytometry. The Journal of Immunology, 175(4), 2357-2365.

Kumar, R., Kumar Pate, D., Prasad, S. K., Sairam, S., & Hemalatha, S. (2011). Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii Thwaites* on streptozotocin-nicotinamide induced type 2 diabetic rats. *Asian Pacific Journal of Tropical Medicine* 904-909.

Kurz, P., Köhler, H., Meuer, S., Hütteroth, T., & Büschenfelde, K. M. Z. (1986). Impaired cellular immune responses in chronic renal failure: Evidence for a T cell defect. *Kidney International 29*, 1209-1214.

Kusaka, Y., Kondou, H., & Morimoto, K. (1992). Healthy lifestyles are associated with higher natural killer cell activity. *Preventive Medicine 21*(5), 602-615.

Laakso, M. (1996). Lipids and lipoproteins as risk factors for coronary heart disease in non insulin dependent diabetes mellitus. *Annals of Medicine, 28*, 341-345.

Lafaille, J. J. (1998). The Role of Helper T Cell Subsets in Autoimmune Diseases. *Cytokine & Growth Factor Reviews 9*(2), 139-151.

Lantz, R. C., Chen, G. J., Sarihan, M., Sólyom, A. M., Jolad, S. D., & Timmermann, B. N. (2007). The effect of extracts from ginger rhizome on inflammatory mediator production. *Phytomedicine 14*(2-3), 123-128.

Larsen, K., Ibrahim, H., Khaw, S. H., & Saw, L. G. (1999). Gingers of Peninsular Malaysia and Singapore. . *Kota Kinabalu: Natural History Publications (Borneo)*, 135.

Li, X., McGrath, K. C. Y., Tran, V. H., Li, Y., Duke, C. C., Roufogalis, B. D., et al. (2013). Attenuation of Proinflammatory Responses by S-[6]-Gingerol via Inhibition of ROS/NF-Kappa B/COX2 Activation in HuH7 Cells. *Evidence-Based Complementary and Alternative Medicine*, 8 pages.

Li, Y., Tran, V. H., Duke, C. C., & Roufogalis, B. D. (2012a). Gingerols of Zingiber officinale Enhance Glucose Uptake by Increasing Cell Surface GLUT4 in Cultured L6 Myotubes. *Planta Medica 78*(14), 1549-1555.

Li, Y., Tran, V. H., Duke, C. C., & Roufogalis, B. D. (2012b). Preventive and Protective Properties of Zingiber officinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid andOtherMetabolic Disorders: ABrief Review. *Evidence-Based Complementary and Alternative Medicine*. Doi: 10.1155/2012/516870.

Li, X., McGrath, K. C. Y., Nammi, S., Heather, A., & Roufogalis, B. D. (2012c). Attenuation of Liver Pro-Inflammatory Responses by Zingiber officinalevia Inhibition of NF-kappa B Activation in High-Fat Diet-Fed Rats. *Basic & Clinical Pharmacology & Toxicology, 110*, 238–244.

Lie, B. A., Todd, J. A., Pociot, F., Nerup, J., Akselsen, H. E., Joner, G., et al. (1999). The predisposition to type 1 diabetes linked to the human leukocyte antigen complex includes at least one non-class II gene. *The American Journal of Human Genetics 64*, 793-800.

Lim, H. W., Hillsamer, P., Banham, A. H., & Kim, C. H. (2005). Cutting edge: direct suppression of B cells by CD4+CD25+ regulatory T cells. *The Journal of Immunology 175*, 4180-4183.

Lu, J., Guan, S., Shen, X., Qian, W., Huang, G., Deng, X., et al. (2011). Immunosuppressive Activity of 8-Gingerol on Immune Responses in Mice. *Molecules 16*, 2636-2645.

Isamy, P., & Subramanian, S. (2011). Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic beta-cell dysfunction in streptozotocin–nicotinamide induced diabetic rats. *Journal of Cellular Physiology 22*, 423-432.

Madkor, H. R., Mansour, S. W., & Ramadan, G. (2011). Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycaemia, dyslipidaemia and oxidative stress in Streptozotocin-nicotinamide diabetic rats. . *British Journal Nutrition 105*, 1210-1217.

Mahendra, P., & Bisht, H. (2011). Diagnostic criteria in different population for diabetes mellitus. *Journal of Pharmacy Research 4*(4), 1189-1192.

Mahluji, S., Ostadrahimi, A., Mobasseri, M., Ebrahimzade Attari, V., & Payahoo, L. (2013). Anti-Inflammatory Effects of Zingiber Officinale in Type 2 Diabetic Patients. Advanced Pharmaceutical Bulletin, 3(2), In press.

Mann, J. I. (2002). Diet and risk of coronary heart disease and type 2 diabetes. *Lancet 360*, 783-789.

Mansoori, D., Jamaati, H. R., Arami, S., Zadsar, M., Abbasian, L., Esteghamati, A. R., et al. (2002). Comparison of Lymphocyte Number and Their Subsets in Patients with Diabetes Mellitus Type II, Tuberculosis and Concomitant TB and Diabetes. *Tanaffos 1*(4), 45-50.

Mansour, M. S., Ni, Y. M., Roberts, A. L., Kelleman, M., Roychoudhury, A., & St-Onge, M. P. (2012). Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: a pilot study. *Metabolism: Clinical and Exprimental 61*(10), 1347-1352.

Mascolo, N., Jain, R., Jain, S. C., & Capasso, F. (1989). Ethnopharmacologic investigation of ginger (Zingiber officinale). *Journal of Ethnopharmacology 27*, 129-140.

Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., et al. (1998). Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes 47*, 224-229.

Mathew, P. T., & Augusti, K. T. (1975). Hypoglycemic effects of onion, Allium cepaLinn. on diabetes mellitus- a preliminary report. *Indian journal of physiology and pharmacology*, *19,* 213-219.

Mensor, L. L., Menezes, F. S., Leitao, G. G., Reis, A. S., Santos, T. S., & Coube, C. S. I. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research 15*, 127-130.

Meydani, S. N., & Han, S. N. (2006). Nutrient Regulation of Immune Response: The case of Vitamin E. In: Present Knowledge in Nutrition. In B. A. Bowman & R. M. Russell (Eds.), *Nutritional Immunity* (Vol. 45, pp. 585-603). Washington, DC.: International Life Sciences Institute.

Millar, D. B., Thomas, J. R., Pacheco, N. D., & Rollwagen, F. M. (1993). Natural killer cell cytotoxicity and T-cell proliferation is enhanced by avoidance behavior. *Brain, Behavior, and Immunity 7*, 144-153.

Mohanty, P., Aljada, A., Ghanim, H., Hofmeyer, D., Tripathy, D., Syed, T., et al. (2004). Evidence for a Potent Antiinflammatory Effect of Rosiglitazone. *Journal of Clinical Endocrinology & Metabolism 89* (6), 2728-2735.

Mojani, M. S., Rahmat, A., Ramasamy, R., & Akhavan Hejazi, S. M. (2012). Palm tocotrienols and α-tocopherol attenuate inflammatory biomarkers in streptozotocin-induced diabetic rats. *Current Topics in nutraceutical Research 10*(2), 117-122.

Morakinyo, A. O., Akindele, A. J., & Ahmed, Z. (2011). Modulation of Antioxidant Enzymes and Inflammatory Cytokines: Possible Mechanism of Anti-diabetic Effect of Ginger Extracts. *African Journal of Biomedical Research 14*, 95-202.

Morimoto, K., Takeshita, T., Nanno, M., Tokudome, S., & Nakayama, K. (2005). Modulation of natural killer cell activity by supplementation of fermented milk containingLactobacillus caseiin habitual smokers. *Preventive Medicine 40*, 589-594.

Morris, D. L., & Komocsar, W. J. (1997). Immunophenotyping Analysis of Peripheral Blood, Splenic, and Thymic Lymphocytes in Male and Female Rats. *Journal of Pharmacological and Toxicological Methods 37*(1), 37-46.

Morrissey, P. J. (1997). *The Immune system: a Functional Perspective* (Vol. 3A): JAI Press Inc.

Murugan, P., & Pari, L. (2007). Influence of tetrahydrocurcumin on erythrocyte membrane bound enzymes and antioxidant status in experimental type 2 diabetic rats. *Journal of Ethnopharmacology 113*, 479-486.

Nicholson, R. L. (1992). Phenolic Compounds and Their Role in Disease Resistance. *Annual Review of Phytopathology 30*, 369-389.

Nirmala, K., Panpatil Virendra, V., Rajkumar, A. K., Bhaskar, V., & K., P. (2012). Dose-dependent effect in the inhibition of oxidative stress and anticlastogenic potential of ginger in STZ induced diabetic rats. *Food Chemistry* 135(4), 2954-2959.

Nurtjahja-Tjendraputra, E., Ammit, A. J., Roufogalis, B. D., Tran, V. H., & Duke, C. C. (2003). Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. *Thrombosis Research 111*, 259-265.

Odebunmi, E. O., Oluwaniyi, O. O., & Bashiru, M. O. (2009). Comparative Proximate Analysis of Some Food Condiments. *Journal of Applied Sciences Research* 1-3.

Ojewole, J. A. (2006). Analgesic, anti-inflammatory and hypoglycaemic effects of ethanol extract of Zingiber officinale (Roscoe) rhizomes (Zingiberaceae) in mice and rats. *Phytotherapy Research 20*, 764-772.

Okamoto, M., Irii, H., Tahara, Y., Ishii, H., Hirao, A., Udagawa, H., et al. (2011). Synthesis of a New [6]-Gingerol Analogue and Its Protective Effect with Respect to the Development of Metabolic Syndrome in Mice Fed a High-Fat Diet. *Journal of Medicinal Chemistry 54*, 6295-6304.

Onyenekwe, P. C., & Hashimoto, S. (1999). The composition of the essential oil of dried Nigerian ginger (Zingiber officinale Roscoe). *European Food Research and Technology 209*, 407-410.

Osuchowski, M. F., Siddiqui, J., Copeland, S., & Remick, D. G. (2005). Sequential ELISA to profile multiple cytokines from small volumes. *Journal of Immunological Methods 302*(1-2), 172-181.

Ozougwu, J. C., & Eyo, J. E. (2011). Evaluation of The Activity of Zingibier Officinale (Ginger) Aqueous Extracts on Alloxan-Induced Diabetic Rats. *Pharmacologyonline 1*, 258-269.

Palsamy, P., & Subramanian, S. (2010). Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic β-cell dysfunction in streptozotocin-nicotinamide-induced diabetic rats. *Journal of Cellular Physiology, 224*(2), 423-432.

Pan, W., Yu, C., Hsuchou, H., Zhang, Y., & Kastin, A. J. (2008). Neuroinflammation facilitates LIF entry into brain: role of TNF. *Cell Physiology: American Journal of Physiology 294*, C1436-C1442.

Parasuraman, S., Raveendran, R., & R., K. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics 1*(2), 87-93.

Pari, L., & Saravanan, R. (2007). Beneficial effect of succinic acid monoethyl ester on erythrocyte membrane bound enzymes and antioxidant status

in streptozotocin–nicotinamide induced type 2 diabetes. *Chemico-Biological Interactions 169*, 15-24.

Park, E.-J., Choi, S.-W., & Cho, S.-H. (2012). Effects of the purified extracts from Lycii Cortex Radicis and ginger on lipid statusand serum cytokine levels in rats fed high fat diet. *Korean Journal of Nutrition* 45(4), 411-419.

Parkin, J., & Cohen, B. (2001). An overview of the immune system. *Lancet, 357*, 1777-1789.

Penna, S. C., Medeiros, M. V., Aimbire, F. S. C., Faria-Neto, H. C. C., Sertié, J. A. A., & Lopes-Martins, R. A. B. (2003). Anti-inflammatory effect of the hydralcoholic extract of Zingiber officinale rhizomes on rat paw and skin edema. *Phytomedicine 10*, 381-385.

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive Protein: a Clinical Update. *Journal of Clinical Investigation* 111(12), 1805-1812.

Phan, P. V., Sohrabi, A., Polotsky, A., Hungerford, D. S., Lindmark, L., & Frondoza, C. G. (2005). Ginger extract components suppress induction of chemokine expression in human synoviocytes. *Journal of Alternative and Complementary Medicine 11*, 149–154.

Pick up, J. C., & Crook, M. A. (1998). Is Type II diabetes mellitus a disease of the innate immune system? *Diabetologia, 41*, 1241-1248.

Pincus, G., & White, P. (1933). On the inheritance of diabetes mellitus. I. An analysis of 675 family histories. *Am. J. Med. Sci., 186*, 1-14.

Pozzilli, P., Zuccarini, O., Iavicoli, M., Andreani, D., Sensi, M., Spencer, K. M., et al. (1983). Monoclonal Antibodies Defined Abnormalities of T-Lymphocytes in Type I (Insulin-dependent) Diabetes. *Diabetes 32*(1), 91-94.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-Reactive Protein, Interleukin 6, and Risk of Developing type 2 Diabetes Mellitus. *Journal of the American Medical Association 286,* 327-334.

Priyarani, M., Padmakumari, K. P., Sankarikutty, B., Lijo Cherian, O., Nisha, V. M., & Raghu, K. G. (2011). Inhibitory potential of ginger extracts against enzymes linked to type 2 diabetes, inflammation and induced oxidative stress. *International Journal of Food Sciences and Nutrition 62*(2), 106-110.

Rahman, A., Tajuddin, & Amin, K. M. Y. (2011). Evaluation of a Unani herbal formulation containingcolchicum, ginger and aloe for anti-inflammatory activity. *Recent Research in Science and Technology 3*(12), 35-39.

Ramadan, G., Al-Kahtani, M. A., & W.M., E.-S. (2011). Anti-inflammatory and Anti-oxidant Properties of Curcuma longa (Turmeric) Versus Zingiber officinale (Ginger) Rhizomes in Rat Adjuvant-Induced Arthritis. *Inflammation, 34*(4), 291-301.

Ramesh, E., Jayakumar, T., Elanchezhian, R., Sakthivel, M., Geraldine, P., & Thomas, P. A. (2009). Green tea catechins, alleviate hepatic lipidemic-oxidative injury in Wistar rats fed an atherogenic diet. *Chemico-Biological Interactions, 180*, 10-19.

Ravindran, P. N., & Nirmal Babu, K. (2005). Ginger the genus Zingiber. *Med. Aromat. Plants-Ind. Profiles, 545*.

Reaven, G. M. (1988). Role of insulin resistance in human disease. *Diabetes Care, 37*, 1595-1607.

Robertson, R. P., Harmon, J., Oanh, P., Tran, T., & Poitout, V. (2004). β-Cell Glucose Toxicity, Lipotoxicity, and Chronic Oxidative Stress in Type 2 Diabetes. *Diabetes, 53*, S119-S124.

Rong, X., Peng, G., Suzuki, T., Yang, Q., Yamahara, J., & Yuhao Li, D. W. (2009). A 35-day gavage safety assessment of ginger in rats. *Regulatory Toxicology and Pharmacology 54*(2), 118-123.

Rozanida, A. R., Nurul Izza, N., Mohd Helme, N. H., & Zanariah, H. A. (2006). Cosmeceutical Product from Species in the Family Zingiberaceae. In M. P. Mazura (Ed.), *Harnessing Cures from Nature: Trends and Prospects* (pp. 31-36). Kepong, Selangor,Malaysia: Forest Research Institute.

Ruzainah, A. J., Ahmad, R. B. A. H., Nor Zaini, C. M., & Vasudevan, R. (2009). Proximate analysis of dragon fruit (Hylecereus polyhizus). *American Journal of Applied Sciences*, 6(7), 1341-1346.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., & Lanzavecchia, A. (1999). Two Subsets of Memory T Lymphocytes with Instinct Homing Potentials and Effector Functions. *Nature, 401*, 708-712.

Sampson, M. J., Winterbone, M. S., Hughes, J. C., Dozio, N., & Hughes, D. A. (2006). Monocyte Telomere Shortening and Oxidative DNA Damage in Type 2 Diabetes. *Diabetes Care, 29*, 283-289.

Sanwal, S. K., Rai, N., Singh, J., & Buragohain, J. (2010). Antioxidant phytochemicals and gingerol content in diploid and tetraploid clones of ginger (Zingiber officinaleRoscoe). *Scientia Horticulturae 124*, 280-285.

Schwertner, H. A., & Rios, D. C. (2007). High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger-containing dietary supplements, spices, teas, and beverages. *Journal of Chromatography B, 856*(1-2), 41-47.

Seino, Y., Nanjo, K., Tajima, N., Kadowaki, T., Kashiwagi, A., Araki, E., et al. (2010). Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Journal of Diabetes Investigation 1*(5), 212-228.

Shane-McWhorter, L. (2001) Biological comple-mentary therapies: a focus on botanical products in diabetes.*Diabetes Spectrum 14*, 199-208.

Shanmugam, K. R., Mallikarjuna, K., Kesireddy, N., & Reddy, K. S. (2011). Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology 49*, 893-897.

Shanmugam, K. R., Mallikarjuna, K., Nishanth, K., Kuo, C. H., & Reddy, K. S. (2011). Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. *Food Chemistry, 124* (4), 1436-1442.

Sheela, C. G., & Augusti, K. T. (1992). Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic Allium sativum Linn. *Indian Journal of Experimental Biology, 30*, 523-526.

Shevach, E. M. (2000). Regulatory T cells in autoimmunity. *Annual Review of Immunology 18*, :423-429.

Singh, A. B., Akanksha, Singh, N., Maurya, R., & Srivastava, A. K. (2009). Anti-hyperglycaemic, lipid lowering and anti-oxidant properties of [6]-gingerol in db/db mice. *International Journal of Medical Sciences 1*(12), 536-544.

Singh, S., & Gupta, A. K. (2013). Evaluation of Phenolics Content, Flavonoids and Antioxidant activity of Curcuma amada (Mango Ginger) and Zingiber officinale (Ginger). *Research and Reviews: Journal of Chemistry, 2*(1), 32-35.

Sitz, K. V., & Birx, D. L. (1999). Lymphocyte Proliferation Assay In M. N. L. & J. H. Kim (Eds.), *Methods in Molecular Medicine* (Vol. 17, pp. 343-353). Totowa, N.J.: Humana Press Inc.

Skzkudelski, T. (2001). The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. *Physiological Research 50*, 536-546.

Subramanian, A., Pushpagandan, P., Ragesekharan, S., Evans, D. A., Latha, P. G., & R., V. (1996). Effect of artemisia pallens wall on blood glucose levels in normal and alloxan – induced diabetic rats. *Journal of Ethnopharmacology 50*, 13-17.

Sweet, M. J., & Hume, D. A. (1996). Endotoxin signal transduction in macrophages. Journal of Leukocyte Biology 60(1), 8-26.

Syed, M. A., Barinas-Mitchell, E., Pietropaolo, S. L., Zhang, Y. J., Henderson, T. S., D.E., K., et al. (2002). Is type 2 diabetes a chronic inflammatory/autoimmune disease? *Diabetes, nutrition & metabolism15*, 68-83.

Takahashi, Y., Tanaka, A., Nakamura, T., Fukuwatari, T., Shibata, K., Shimada, N., et al. (2004). Nicotinamide suppresses hyperphosphatemia in hemodialysis patients. *Kidney International 65*, 1099-1104.

Tanabe, M., Chen, Y. D., Saito, K., & Kano, Y. (1993). Cholesterol biosynthesis inhibitory components from Zingiber officinale Roscoe. *Chemical Pharmacy Bulletin 41*, 710 -713.

Tee, E. S., Rajam, K., Young, S. I., Khor, S. C., Zakiyah, H. O. (1996). Laboratory procedures in nutrient analysis of foods. pp. 4-10. Malaysia: Division of Human Nutrition, Institute for the Medical Research, Kuala Lumpur.

Tejasari. (2007). Evaluation of Ginger (Zingiber officinale Roscoe) Bioactive Compounds in Increasing the Ratio of T-cell Surface Molecules of CD3+CD4+:CD3+CD8+ In Vitro. *Malaysian Journal of Nutrition 13*(2), 161-170.

Tejasari, Rungkat-Zakaria, F., & Sajuthi, D. (2006). Ginger (Zingiber officinale Roscue) Root Bioactive Compounds Increased Cytolitic Response of Natural Killer (NK) Cells Against Leucemic Cell Line K-562 in vitro. *Jurnal Teknol. dan Industri Pangan 17*(2), 97-108.

Thomson, M., Al-Qattan, K. K., Al-Sawan, S. M., Alnaqeeb, S. M., Khan, I., & Ali, M. (2002). The use of ginger (Zingiber officinale Rosc.) as a potential anti-inflammatory and antithrombotic agent. . *Prostaglandins, Leukotrienes and Essential FattyAcids 67*(6), 475-478.

Trapani, J. A., & Smyth, M. J. (2002). Functional Significance of The Perforin/Granzyme Cell Death Pathway. *Nature Review Immunology 2*, 735-747.

Tsujino, M., Kinpara, I., Nakamura, T., Suda, T., Saitou, Y., Kudou, H., et al. (1996). Peripheral lymphocyte subset of patients with pancreatic diabetes mellitus--about a decreased ratio of T-LGL and ability of host defense. *Kansenshogaku Zasshi 70*(4), 325-330.

Uchida, K., Takase, H., Nomura, Y., Nakamura, Y., Igimi, H., Takasu, N., et al. (1994). Development of Atherosclerosis in Alloan Diabetic Rats. *Journal of Atherosclerosis Thrombosis, 1*(2), 118-128.

USDA Nutrient Database. National Nutrient Database for Standard Reference Release 25 (Publication no. http://ndb.nal.usda.gov/ndb/foods/show/2954). Retrieved 04 July 2013.

Valentine, F., & Lederman, H. (2000). Lymphocyte Proliferation Assay (LPA) *ACTG Laboratory Technologist Committee*, 1-11.

van Oostrom, A. J., J. P. van Wijk, T. P. Sijmonsma, T. J. Rabelink & M. Castro Cabezas, 2004: Increased expression of activation markers on monocytes and neutrophils in type 2 diabetes. *Netherlands Journal of Medicine 62*, 320-325.

Watson, J. V. (2004). *Introduction to Flow Cytometry*. New York: Press Syndicate of the University of Cambridge.

Wilasrusmee, C., Kittur, S., Siddiqui, J., Bruch, D., Wilasrusmee, S., & Kittur, D. S. (2002). In Vitro Immunomodulatory Effects of Ten Commonly Used Herbs on Murine Lymphocytes *8*(4), 467-475.

Wilasrusmee, C., Siddiqui, J., Bruch, D., Wilasrusmee, S., Kittur, S., & Kittur, D. S. (2002). In vitro immunomodulatory effects of herbal products. *The American Surgeon* 68, 860-864.

Wohlmuth, H., Leach, D. N., Smith, M. K., & Myers, S. P. (2005). Gingerol content of diploid and tetraploid clones of ginger (Zingiber officinale Roscoe). *Journal of Agricultural and Food Chemistry 53*, 5772–5778.

Yeh, S.-H., Chuang, H., Lin, L.-W., Hsiao, C.-Y., Wang, P.-W., Liu, R.-T., et al. (2009). Regular Tai Chi Chuan exercise improves T cell helper function of patients with type 2 diabetes mellitus with an increase in T-bet transcription factor and IL-12 production. *British Journal of Sports Medicine 43*, 845-850.

Zakaria-Rungkat, F., Nurahman, E., & Tejasari. (2003). Antioxiadat and Immunoenhacement activities of Ginger (zingiber Officinale Roscoe) Extracts and Compounds in In vitro and In vivo Mouse and Human System. *Nutraceuticals and Food 8*, 96-104.

Zhang, H., Yu, D., & Chen, Y. (2002). Study on the Changes in Immune Function and Its Correlation with the Levels of Blood Glucose and Insulin in Patients with Type 2 Diabetes Mellitus. *Labeled Immunoassays and Clinical Medicine 2002,02*.

Zhang, L., Kamanna, V. S., Zhang, M. C., & Kashyap, M. L. (2008). Niacin inhibits surface expression of ATP synthase β chain in HepG2 cells: implications for raising HDL. *Journal of Lipid Research 49*, 195-1201.

Zhang, L., Zhang, Y., Xia, Q., Zhao, X. M., Cai, H. X., Li, D. W., et al. (2008). Effective control of blood glucose status and toxicity in streptozotocin-induced diabetic rats by orally administration of vanadate in an herbal decoction. *Food and Chemical Toxicology* , 46 (9), 2996-3002

Zhang, X. W. T., Iwaoka, W. T., Huang, A. S., Nakamoto, S. T., & Wong, R. (1994). Gingerol Decreases after Processing and Storage of Ginger. *Journal of Food Science 59*(6), 1338-1340.

Zhao, D.-M., Thornton, A. M., DiPaolo, R. J., & Shevach, E. M. (2006). Activated CD4+CD25+ T cells selectively kill B lymphocytes. *Blood, 107*, 3925-3932.

Zhen, Y., Sun, L., Liu, H., Duan, K., Zeng, C., Zhang, L., et al. (2012). Alterations of peripheral CD4+CD25+Foxp3+ T regulatory cells in mice with STZ-induced diabetes. *Cellular & Molecular Immunology, 9*, 75-85.

Zhou, H., Deng, Y., & Xie, Q. (2006). The modulatory effects of the volatile oil of ginger on the cellular immune response in vitro and in vivo in mice. *Journal of Ethnopharmacology 105*, 301-305.