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
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and other art works, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
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-$\alpha$ 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 Streptozocin-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 streptozocin (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 signifikan (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-\(\alpha\) 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: 13 December 2013
<table>
<thead>
<tr>
<th>CHAPTER</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>2.1 Ginger</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2.1.1 Reviews of Ginger</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2.1.2 Ginger Family and Components</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2.1.3 Potential Benefits of Ginger</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2.1.4 Antioxidant Capacity of Ginger Rhizome</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2.2 Diabetes Mellitus</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2.2.1 Pathophysiology</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2.2.2 Classification</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2.3 Significance of Herbal Medicine in Diabetes and Hyperglycemia</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2.4 Ginger, High Blood Glucose and Dyslipidemia</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2.4.1 Anti-diabetic Mechanism of Ginger Rhizome</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2.5 Overview of the Immune system</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2.5.1 Innate Response</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2.5.2 Specific Immunity</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2.5.3 Immune cells</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2.5.4 Evaluation of Immune System Function</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>2.6 Diabetes Mellitus and Immune system</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>2.7 Insulin and Inflammation</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>2.8 Ginger, Inflammation and Immune Function</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
2.9 Laboratory Methods
  2.9.1 Diabetes induction in rat model
  2.9.2 Cytokine Assay
  2.9.3 Lymphocyte Proliferation Assay
  2.9.4 Immunophenotyping Assay

2.10 Summary

3 METHODOLOGY
  3.1 Characteristic of Ginger Rhizome Powder
    3.1.1 Sample Preparation
    3.1.2 Freeze-drying Method
    3.1.3 Proximate analysis
    3.1.4 Determination of Active Compounds
    3.1.5 Determination of Total Flavonoids (TF) and Total Phenolic Content (TPC)
    3.1.6 Antioxidant Activities: DPPH Radical Scavenging
  3.2 In vivo study
    3.2.2 Induction of Diabetes
    3.2.3 Treatment Schedule
    3.2.4 Body Weight and Food Intake
    3.2.5 Blood Collection
    3.2.6 Biomedical Analysis
  3.3 In vitro study
    3.3.1 Spleen Proliferation Assay
    3.3.2 Lymphocyte Immunophenotyping assay

3.4 Statistical Analysis

4 RESULTS
  4.1 Characteristics of Ginger Rhizome
    4.1.1 Moisture Content, Yield and Nutritional Composition
    4.1.2 Determination of Active Components
    4.1.3 Total Flavonoids and Phenolic Contents
    4.1.4 DPPH Radical Scavenging Activity
  4.2 In vivo Study
    4.2.1 The Effects of Ginger on Body Weight and Food
Intake of Healthy and Diabetic Rats
4.2.2 Effects of Ginger on whole and Plasma Glucose on Healthy and Diabetic Rats
4.2.3 The Effects of Ginger on Plasma Lipid Profile and Atherogenic Indices of Healthy and Diabetic Rats
4.2.4 The Effects of Ginger on C-reactive Protein of Healthy and Diabetic Rats
4.2.5 The Effects of Ginger on Interleukin-6 of Healthy and Diabetic Rats
4.2.6 The Effects of Ginger on Tumor Necrosis-α of Healthy and Diabetic Rats
4.3 In vitro Study: Immunological Effects of Ginger on Rats
4.3.1 Activation of Spleen T cells
4.3.2 Immunophenotyping Assay
4.4 Relationship between Different Variables of Metabolic Indexes and Immunological Factors

5 DISCUSSION
5.1 Ginger Rhizome: Constituents and Characteristics
5.2 In vivo Study
5.2.1 Evaluation Metabolic and Immunological Changes Following Diabetes Induction by Nicotinamide and Streptozotocin
5.2.2 Body Weight and Food Intake
5.2.3 Fasting Blood Glucose
5.2.4 Lipid Profile and Atherogenic Indexes
5.2.5 Cytokines Changes
5.3 In Vitro Study
5.3.1 Proliferation Assay
5.3.2 Immunophenotyping Assay

6 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH
REFERENCES/ BIBLIOGRAPHY 111
APPENDICES 131
BIO DATA OF STUDENT 143
LIST OF PUBLICATIONS 143

xvi
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Previous Studies on Benefits of Ginger Rhizome and Extracts</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Previous Studies on Anti-inflammatory Effects of Ginger in Rodent Model and Cell Line</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Gradient Program of HPLC</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Groups of Study, Diet and Treatment</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>Nutritional Composition of Ginger Rhizome</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Daily Doses of Bioactive Intakes in Ginger Treated Rats</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>DPPH Scavenging Activities of the Methanolic Extract of Ginger Rhizome at Concentration of 50 µg/ml</td>
<td>54</td>
</tr>
<tr>
<td>8</td>
<td>Levels of Baseline Plasma Lipid Profile in Normal and Diabetic Rats</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>Levels ofMarkers Lipid Profile (mmol/L) in Normal and Diabetic Rats Treated with Ginger</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>Comparing Lymphocyte Population in Normal and Diabetic Control Rats</td>
<td>74</td>
</tr>
<tr>
<td>11</td>
<td>Correlation and Multiple Regression Analysis of the Relationship between Final Body Weight and Other Factors</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td>Correlation among Lipid Profile Markers and Multiple Regression Analysis between Blood Glucose and Triglyceride</td>
<td>86</td>
</tr>
<tr>
<td>13</td>
<td>Relationship between Blood Glucose Level and Immunologic Factors</td>
<td>86</td>
</tr>
<tr>
<td>14</td>
<td>Associations between Responses of Spleen cells to the Mitogens and Lymphocyte Subsets</td>
<td>88</td>
</tr>
</tbody>
</table>

# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>xvii</td>
</tr>
</tbody>
</table>
1 Structure of Active Components  
2 Related Mechanism of Glucose Lowering Effect of Ginger and Protection for Diabetic Complications  
3 Immune System Cells  
4 Responses of B cells and T cells in Adaptive Immunity  
5 Role of Cytokines and Innate Immune System in the Etiology of Type 2 Diabetes.  
6 Structure of Streptozotocin (a) and Nicotinamide (b)  
7 Principal of Sandwich ELISA  
8 Study Design  
9 Percentage of Active Components for Gingerols and Shogaols  
10 UV Chromatogram of Control (a), Standard sample (b) and Ginger Rhizome (c).  
11 DPPH Radical Scavenging Activity of the Methanolic Extracts of Ginger Rhizome Compared with BHT and α-tocopherol  
12 Body Weight Trends of Healthy Rats during the Treatment Period  
13 Body Weight Trends of Diabetic Rats during the Treatment Period  
14 Comparing Body Weight Changes before (day 0) and after Treatment (day 42) in Healthy and Diabetic Rats  
15 Mean Intake of Food in Healthy and Diabetic Rats  
16 Levels of Whole Blood Glucose in Normal and Diabetic Rats  
17 Levels of Plasma Glucose before and after Treatment  
18 Comparing Changes of Total Cholesterol level after 6 weeks of Treatment in Groups of Study  
19 Comparing Changes of Triglyceride level after 6 weeks of Treatment in Groups of Study  
20 Comparing Changes of LDL-c Level after 6 weeks of Treatment in Groups of Study  

xviii
21 Comparing Changes of HDL-c level after 6 weeks of Treatment in Groups of Study
22 Atherogenic Index (1) in Normal and Diabetic Rats Treated with Ginger
23 Atherogenic Index (2) in Normal and Diabetic Rats Treated with Ginger Rhizome
24 Levels of C-reactive Protein before and after Treatment
25 Levels of Interleukin-6 before and after Treatment
26 Levels of TNF-α before and after Treatment
27 Assessment of Splenocytes Proliferation using 1 μg/ml PHA and LPS
28 Assessment of Splenocytes Proliferation using 5 μg/ml PHA and LPS
29 Assessment of Regulatory T cells (%CD4⁺CD25⁺) in Normal and Diabetic Treated Rats
30 Assessment of Helper T cells (%CD3⁺CD4⁺) in Normal and Diabetic Treated Rats
31 Percentage of CD4⁺ cells in Normal and Diabetic Treated Rats
32 CD4⁺CD25⁺ Expression among Normal Groups
33 CD4⁺CD25⁺ Expression among Diabetic Groups
34 Assessment of Percentage T cells (CD3⁺) Expression
35 Percentage of B cells (CD45ra⁺) Expression in Normal and Diabetic Rats
36 Percentage of Natural Killer cell (CD161a⁺) Expressions in Normal and Diabetic Rats
37 CD45ra⁺ Expression among Normal and Diabetic Rats
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
AlCl₃ 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
°C Degree Centigrade
12-DHGB 12-Dehydrogingerdione
dl Deciliter
DPPH Diphenylpicryl-hydrazyl
DQ αβ heterodimer of the MHC Class II type
DTH Delayed Type Hypersensitivity
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

xx
GDM  Gestational Diabetes Mellitus
Glut-4  Glucose transporter type 4
HDL-c  High Density Lipoprotein Cholesterol
HF  High Fat
HLA-DP  \( \alpha, \beta \) 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-\( \gamma \)  Interferon-\( \gamma \)
I\( \kappa \)B  Inhibitor Kappa-B
IL-6  Interleukin-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
\( \mu \)g  Microgram
\( \mu \)l  Microliter
mmol  Milli mol
MHC class  Major Histocompatibility Complex Class
mM  Milli mol
MMP-9  Metallo proteinase-9
MNCs  Mononuclear 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
NF\( \kappa \)B  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-Fernaíndez, 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 \( \alpha \)-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

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

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

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

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

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

HA 6: Ginger improves lymphocyte subpopulations in the streptozotocin-nicotinamide induced diabetic rats.
REFERENCES/ BIBLIOGRAPHY


