



UNIVERSITI PUTRA MALAYSIA

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

MANSOOREH SADAT MOJANI QOMI

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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

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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

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December 2013

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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 $51.4 \pm 0.4\%$ 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 $\mu\text{g/ml}$), 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 respond. Once the levels of stimuli increased to 5 $\mu\text{g/ml}$, 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 $\text{CD4}^+\text{CD25}^+$ 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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

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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 $51.4 \pm 0.4\%$ 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 ($1 \mu\text{g/ml}$), dan sel T bagi kedua-dua keadaan iaitu tidak dirangsang ($p = 0.001$) dan dirangsang ($p = 0.031$ untuk LPS dan $p = 0.001$ untuk PHA) telah gagal untuk bertindak secara signifikan. Apabila paras perangsang ditingkatkan kepada $5 \mu\text{g/ml}$, 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 $\text{CD4}^+\text{CD25}^+$ (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 $1 \mu\text{g/ml}$ 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 $5 \mu\text{g/ml}$ menghasilkan satu proliferasi besar dalam semua kumpulan rawatan termasuklah *glibenclamide* ($p < 0.05$); sebaliknya 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 suplementasi 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.

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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.

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ABCC8	ATP-binding cassette transporter sub-family C member 8
Abs	Absorbance
ACTH	Adrenocorticotrophic 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
°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 isothiocyanate
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-dimer 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
I κ 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
μ g	Microgram
μ 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 κ 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
PPAR γ	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-Fernández, 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.

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