



Ain Shams University
Faculty of Science
Biochemistry Department

Biochemical Study on the Effect of Coffee and Cinnamon on the Streptozotocin induced Diabetes in Rats.

A thesis

“Submitted for the degree of Master of Science As a partial fulfillment for requirements of the master of science in Biochemistry”

Submitted by

Rabab Abd El-Wahab Saad Abdel Hamid
(B.Sc.in Biochemistry 2010)

Under the supervision of

Prof. Dr. Fatma Farag Abdel Hamid

Professor of Biochemistry
Faculty of Science
Ain Shams University

Prof. Dr. Dawoud Fakhary Habib

Research Professor of Medical Biochemistry
Medical Biochemistry Department
National Research Center

Dr. Marwa Galal El-deen Abdo Hegazy

Lecturer of Bio chemistry
Faculty of Science
Ain Shams University

2016

Acknowledgements

Firstly and foremost, thanks to **ALLAH**, who give me everything in my life, and I supplicate **Allah** to make my life in a perfect way.

I wish to express my appreciation to **Prof. Dr. Fatma Farag Abdel Hamid**, Professor of Biochemistry, Faculty of Science, Ain Shams University, for constant encouragement and offering of facilities.

I express my deepest gratitude and appreciation to **Prof. Dr. Dawoud Fakhary Habib**, Research Professor of Medical Biochemistry, Medical Biochemistry Department, National Research Center for giving me the honor of working under him supervision, for him valuable help and guidance throughout the whole work and above all for him in for gettable sincere encouragement. I wish also to record my deepest gratitude.

I wish to express my appreciation to **Dr. Marwa Galal El-deen Abdo Hegazy**, Lecturer of Bio chemistry, for his much valued guidance, valuable advice and assistance during the work in this thesis and his effective supervision which were greatly beneficial and helpful for me.

I express my deepest gratitude and appreciation to **Prof. Dr. Zakarya El- Khyad**, Research Professor of Medical Biochemistry,

Medical Biochemistry Department, National Research Center for his continuous helpful and encouragement me.

I am deeply grateful to **Dr. Nadia Mohammed Ahmed Mohammed** assistant professor, Medical Biochemistry department, National Research center, for sound advice given throughout the course of the present work and during the written of the thesis.

I would like to express my deep thanks and gratitude to **Dr. Hazem**, assistant researcher of community Medicine department, National Research Center for him supports in statistical analysis and encouragement me.

I wish also to convey a meaning message of gratitude to my Mother, she consider for me Mother and Father at the same time and everything in my life, for her voluble help and guidance throughout my whole life and I don't want anything in my life except her satisfaction.

Finally, my deep thanks to father, to all deep members in my family and my friends for their continuous encouragement, help and patients

I declare that this thesis has been composed by me and the work of which is a record has been done by me. It has not been submitted for a degree at this or any other university.

Rabab Abdel Wahab

List of content

<i>Title</i>	<i>Page</i>
Abstract	I
List of abbreviations	II
List of figures	V
List of tables	VIII
1.Introduction	1
Aim of work	4
2. Review of literature	5
2.1.Diabetes mellitus	5
2.1.1. Diabetes mellitus and clinical implications	5
2.1.2.Etiological classification of diabetes mellitus	7
2.1.2.1.Type 1 diabetes	8
2.1.2.1.1. Immune-mediated diabetes	8
2.1.2.1.2.Idiopathic diabetes	10
2.1.2.2. Type 2 diabetes	11
2.1.2.3.Gestational diabetes mellitus (GDM)	13
2.1.2.4. Specific type of diabetes due to other causes	14
2.1.2.4.1. Monogenic diabetes syndromes	14
2.1.2.4.1.1 Neonatal diabetes	14
2.1.2.4.1.2 Maturity-onset diabetes of the young (MODY)	15
2.1.2.4.2.Cystic fibrosis-related diabetes (CFRD)	15
2.1.2.4.3. Experimental models of diabetes	17
2.1.3.Complications	17
2.1.3.1.Macrovascular disease	18
2.1.3.1.1.Cardiovascular disease(CVD)	18
2.1.3.2.Microvascular complications	19
2.1.3.2.1.Nephropathy	19
2.1.3.2.2. Retinopathy	19
2.1.3.2.3. Neuropathy	20
2.2. Insulin	21
2.2.1.Insulin resistance	23
2.2.2.Insulin receptor and mechanism of action	24

2.3.Oxidative Stress	26
2.3.1. Antioxidant	29
2.3.2. Biological action of antioxidants	35
2.3.3.The mechanism of oxidative stress in diabetic patients	37
2.3.3.1. Increased polyol pathway flux	40
2.3.3.2. Increased intracellular AGEs formation	43
2.3.3.2.1.Intracellular production of AGEs precursors can damage cells by 3 general mechanisms	44
2.3.3.3. Activation of protein kinase C	46
2.3.3.4. Increased flux through the hexosamine pathway	48
2.4.Herbal medicine and diabetes	49
2.4.1.Cinnamon	51
2.4.1.1.Effect of cinnamon on insulin	54
2.4.1.2. Effect of cinnamon on glucose and lipid profile	58
2.4.1.3. Effect of cinnamon on oxidative stress	61
2.4.1.4. Effect of cinnamon kidney function	64
2.4.1.5. Active components in cinnamon aqueous extract	67
2.4.2.Coffee	69
2.4.2.1. Effect of coffee on insulin	70
2.4.2.2. Effect of coffee on glucose & lipid profile	72
2.4.2.3. Effect of coffee on oxidative stress	73
2.4.2.4. Effect of coffee on kidney function	75
2.4.2.5.Active component in coffee	76
2.4.2.6.Changes in chlorogenic acids composition of coffee beans during processing	78
3. Materials & methods	80
3.1Materials	80
3.1.1.Experimental animals	80
3.1.2 Chemicals	80
3.2.Methods	81
3.2.1.Induction of diabetes	81
3.2.2.Preparation of natural compounds extracts	81
3.2.3. Experimental design	82

3.2.4. Blood collection	83
3.3. Biochemical assay	84
3.3.1. Determination of fasting blood glucose	84
3.3.2. Determination of serum insulin level	85
3.3.3. Insulin resistance	89
3.3.4. Determination of serum total cholesterol	90
3.3.5. Determination of serum triacylglycerols	92
3.3.6. Determination of serum high density lipoprotein cholesterol (HDL-cholesterol)	94
3.3.7. Determination of low density lipoprotein-cholesterol (LDL-c)	97
3.3.8. Determination of very low density lipoprotein-cholesterol (VLDL-c)	98
3.3.9. Determination of serum blood urea nitrogen	98
3.3.10. Determination of serum creatinine	100
3.3.11. Determination of serum uric acid	101
3.3.12. Determination of serum nitric oxide	103
3.3.13. Determination of serum reduced glutathione	104
3.3.14. Determination of serum paraoxinase	106
3.3.15. Determination of serum malondialdehyde	107
3.4. Statistical methods	109
4. Results	110
5. Discussion	181
6. Summary	198
7. References	203
8. Arabic summary	

Abstract

This work was done to study the effect of black, green coffee and cinnamon on the level of glucose, insulin, and lipid profile and antioxidant status in control and diabetic rats which received black and green coffee and cinnamon separately or together in double or triple combinations. The study focused to deduce the best method of herbal treatment alone or mix herbs double or triple. The statistical analysis of the results showed that insulin, HDL, reduced glutathione (GSH) and paraoxinase (PON) were increased in triple combination treated groups than either in double combination or single treated groups. In contrast, glucose, total cholesterol, triacylglycerols (TAG), insulin resistance (HOMA IR), malondialdehyde (MDA) and nitric oxide (NO) were decreased in triple combination treated group either double combination than single treated groups while there was no effect on urea and creatinine in treated groups compared to diabetic control group. This study recommends use the triple combination treatment by cinnamon, green and black coffee due to its anti-hyperglycaemia, antihyperlipidemia and antioxidant effects.

List of abbreviations

AGEs	Advanced Glycation End products
AIDS	Acquired Immunodeficiency Syndrome
ATP	adenosine-5-triphosphate
CAE	Cinnamon Aqueous Extract
CAN	Cardiovascular autonomic neuropathy
CAT	Catalase
CE	Cholesterol Estrase
CFRD	Cystic Fibrosis-Related Diabetes
CGA	Chlorogenic Acid
CHD	Coronary Heart Disease
CISI	Composite Index of Insulin Sensitivity
CO	Cholesterol Oxidase
CVD	Cardiovascular disease
DAG	Diacycle glycerol
DEA-HCl/AAP	N,N diethylaniline-HCl/4-aminoantipyrine
DECAF	a Decaffeinated Coffee Extract
DIFEQ	Quinide, 3,4-diferuloyl-1,5-quinolactone
DM	Diabetes Mellitus
DPN	Diabetic peripheral neuropathy
DTNB	5, 5 dithiobis-2- nitrobenzonic acid
eNOS	Endothelial Nitric Oxide Synthase Manganese
ESRD	End-Stage Renal Disease
FA	fatty acid
FBG	Fasting Blood Glucose
FBS	Fasting Blood Sugar
FFA	Free Fatty Acid
FR	Free Radicals
GAD	Generalized Anxiety Disorder
GDM	Gestational diabetes mellitus
GFAT	glutamine: fructose-6 phosphate amidotransferase

GI	Glycemic Index
GIP	Glucose dependent insulinotropic polypeptide
GK	Glycerol kinase
GLDH	glutamate dehydrogenase
GLP-1	Glucagon-like peptide-1
Glut2 receptor	Glucose transporter 2 receptor
GOD	glucose oxidase
GPO	Glycerol-3-phosphate-oxidase
GSH	Reduced Glutathione
GST	Glutathione Transferase
HbA1c	Glycated hemoglobin
HCL	Hydrochloric acid
HepG2	Human liver cancer cell line
HFD	High Fructose Diet
HIF-1	Hypoxia-inducible factor
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMG-CoA reductase	3-hydroxy-3-methyl-glutaryl-CoA reductase
HNF-1a	Hepatocyte Nuclear Factor
HOMA	Homeostasis Model Assessment
HPLC	High performance liquid chromatography
HPO	horseradish peroxidase
HSDA	sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline
IGF1R	Insulin like Growth Factor-1 receptor
IGT	Impaired glucose tolerance
InsR	Insulin Receptor
(IRS1)	IR substrate-1
(IPF)-1	Insulin Promoter Factor
LDL	Low-Density Lipoprotein
LPL	lipoprotein lipase
MDA	Malonaldehyde

MnSOD	Manganese Superoxide Dismutase
MODY	Maturity-Onset Diabetes of the Young
NADH	reduced nicotinamide-adenine dinucleotide
NEDA	N- (1 – naphthyl) – ethylenediamine
NO	Nitric oxide
OGTT	oral glucose tolerance test
OPs	OrganoPhosphates
PAP	peroxidase
PEG	polyethylene glycol
PI3K	phosphoinositide 3-kinase
PKC	protein kinase C
POD	peroxidase
PON	paraoxonase
RAGEs	Receptor for Advanced Glycation End products
RBP4	Retinol-Binding Protein 4
RM	Reactive Metabolites
RONS	Reactive oxygen nitrogen species
ROS	Reactive Oxygen Species
RTK	Receptor Tyrosine Kinase
SDH	sorbitol dehydrogenase
SOD	Super Oxide Dismutase
STZ	Streptozotocin
TCA	Trichloric acetic acid
TGF- β	Transforming growth factor-beta
TMB	Tetramethylbenzidine
UACR	Urine Albumin-to-Creatinine Ratio
UDP	Uridine Diphosphate
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization
ZnT8	Zinc Transporter 8

List of figures

<i>Fig.</i>	<i>Title</i>	<i>page</i>
1	The regulation of metabolism by insulin.	22
2	Structure of insulin receptors.	25
3	Insulin signaling pathways that regulate glucose metabolism in muscle cells and adipocytes.	26
4	Over nutrition and decreased physical activity lead to increased glucose and free fatty acid (FFA) loads in cells.	27
5	Mutual association between oxidants and antioxidants	36
6	The mechanism of oxidative stress in diabetic patients.	38
7	Oxidative stress and diabetes complications.	40
8	Mean value of FBS levels (mg/dl) before treatment in different studied groups.	112
9	Mean value of fasting blood glucose levels (mg/dl) after treatment in different studied groups.	115
10	Blood glucose changes (%) after treatment in different studied groups.	118
11	Mean value of insulin levels (μ IU/L) after treatment in different studied groups.	122
12	Mean value of insulin resistance levels after treatment in different studied groups.	126
13	Mean value of serum total cholesterol levels (mg/dl) after treatment in different studied groups.	130
14	Mean value of serum triacylglycerol (mg/dl) after treatment in different studied groups.	133
15	Mean value levels of serum HDL-c (mg/dl) after treatment in different studied groups.	137