Biochemical Studies on Extracts of Some Plants to Ameliorate Metabolic Syndrome Disorders

Presented by

Rania Sayed Taha Mohammed
Assistant Researcher- Agriculture Research Center
M.Sc. of Science- Cairo University- 2016

A Thesis Submitted To
Faculty of Science-Cairo University

In Partial Fulfillment of the
Requirements for the Degree of

Ph.D. of Science
(Biochemistry)

Chemistry Department
Faculty of Science
Cairo University
2021

ABSTRACT

Student Name: Rania Sayed Taha Mohammed

Title of the thesis: **Biochemical studies on extracts of some plants to ameliorate metabolic syndrome disorders**

Degree: Ph.D. Of Science (Biochemistry)

The purpose of this research was to determine how aqueous and ethanolic extracts of Nigella Sativa seeds and mulberry leaves affected metabolic syndrome. To fulfill the target of this study, a total of 36 rats were selected. They were split into six groups: Group 1 is the control group, whereas Group 2 is the induction group which have been fed on high fat diet supplemented with cholesterol powder and fructose in drinking water to induce metabolic syndrome, Group 3:ethanolic Nigella Sativa extract treated group, Group 4: aqueous Nigella Sativa extract treated group, Group 5:ethanolic mulberry leaves extract treated group, Group 6: aqueous mulberry leaves extract treated group. Results obtained from this study showed significant improvement in metabolic disorders in groups of rats treated with either ethanolic or aqueous extracts of Nigella Sativa seeds and mulberry leaves as compared with induction group. The antioxidant activity of phenolic acids and flavonoids of both plants extracts in addition of anthocyanin content of mulberry extracts are responsible for this improvement. The results of histopathology revealed that there was damage in the heart, pancreas and liver in induction group. This damage was improved in treated groups with ethanolic and aqueous extracts.

Keywords: Metabolic syndrome-High fat diet and fructose-Nigella Sativa-Mulberry leaves

Supervisors: **Signature:**

1- Prof. Dr. Mohammed Ali El-Desouky

2- Dr. Hala Aly Thabet

Prof. Dr. Tayseer Abdel khalek Abdallah

Chairman of Chemistry Department

Faculty of Science- Cairo University

LIST OF CONTENTS

Title	Page
APPROVAL SHEET	
ACKNOWLEDGEMENT	
ABSTRACT	
CONTENTS	I
LIST OF ABBREVIATIONS	II
LIST OF TABLES	IV
LIST OF FIGURES	VI
INTRODUCTION AND AIM OF THE WORK	1
REVIEW OF LITERATURE	6
MATERIALS AND METHODS	24
RESULTS	43
DISCUSSION	82
SUMMARY & CONCLUSION	102
REFERENCES	108
ARABIC SUMMARY	1

LIST OF ABBREVIATION

Abbreviation	Meaning
MS	Metabolic Syndrome
HPLC	High Performance Liquid Chromatography
H.F.D	High Fat Diet
TFC	Total Flavonoid Content
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
AST	Aspartate Transaminase
ALT	Alanine Transaminase
INS	Insulin
O.D	Optical Density
HOMA-IR	homeostasis model assessment index for insulin resistance
TC	Total Cholesterol
TG	Triglycerides
GLUT5	Glucose transporter 5
CHD	Coronary Heart Disease
CVD	Cardiovascular Atherosclerotic Diseases
DMT2	Diabetes Mellitus Type 2
apoB	Apolipoprotein B
BP	Blood Pressure
IR	Insulin Resistance
PUFA	Polyunsaturated Fatty Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl

VLDLs	Very low density lipoproteins
DAG	Diacyl glycerol
PKC	protein kinase c
IRS1	insulin receptor substrate 1
ROS	Reactive oxygen species
LPL	Lipoprotein lipase
NAFLD	Nonalcoholic fatty liver disease
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
CCl4	Carbon tetra chloride
STZ	Streptozotocin
CKMB	Creatine kinase isoenzyme MB
AMPK	Adenosine monophosphate-activated protein
Glut4	Glucose transporter 4
NO	Nitric oxide
MLE	Mulberry leaves extracts
NS	*Nigella Sativa*

LIST OF TABLES

Table No.	Title	Page No.
1	Composition of basal diet and high-fat diet* (g/100g)	**29**
2	Ingredients of salt mixture in basal diet	**30**
3	Ingredients of vitamin mixture in basal diet.	**30**
4	Concentration of phenolic compounds (µg/mg) in different *Nigella sativa* seeds extracts	**44**
5	Concentration of phenolic compounds (µg/mg) in different mulberry leaves extracts	**45**
6	Concentration of flavonoids components (µg/mg) in different *Nigella sativa* seeds extracts	**46**
7	Flavonoids components in ethanolic and aqueous mulberry leaves extracts.	**47**
8	DPPH (%) activity of different studied extracts	**48**
9	Concentration of total anthocyanin (mg/100g) in ethanolic and aqueous extracts of mulberry leaves	**49**
10	Concentration of cyanidine (%) in ethanolic and aqueous extracts of mulberry leaves	**49**
11	Total polyphenols mg (GAE/100g) in plant studied extracts	**50**
12	Total flavonoids (mg RE /100g) in plant studied extracts	**50**
13	Comparison between negative and positive groups in all biochemical parameters	**51**
14	Effect of studied plants extracts on serum cholesterol concentrations (mg/dL) in induced rats	**61**
15	Effect of studied plants extracts on serum triglycerides concentrations (mg/dL) in induced rats	**62**
16	Effect of studied plants extracts on serum HDL-C concentrations (mg/dL) in induced rats	**63**

17	Effect of studied plants extracts on serum LDL-C concentrations (mg/dL) of induced rats	**64**
18	Effect of studied plants extracts on serum glucose concentrations (mg/dL) of induced rats	**68**
19	Effect of studied plants extracts on serum insulin concentrations (ng/ml) of induced rats	**69**
20	Effect of studied plants extracts on insulin resistance (HOMA-IR) of induced rats	**70**
21	Effect of studied plants extracts on serum ALT activity (IU/L) of induced rats	**73**
22	Effect of studied plants extracts on serum AST activity (IU/L) in induced rats	**75**
23	Effect of studied plants extracts on blood pressure (mmHg) in induced rats	**78**

LIST OF FIGUARS

Figure No.	Title	Page No.
1	Mean insulin concentration (ng/mL) in negative control and positive control groups	**52**
2	Mean glucose concentration (mg/dL) in negative control and positive control groups	**52**
3	Pancreas of rat from negative control showing no histopathological changes (H & E X 400)	**53**
4	Pancreas of rat from positive control showing congestion of pancreatic blood vessel (H & E X 400)	**53**
5	Mean triglycerides concentration (mg/dL) in negative and positive control groups	**54**
6	Mean cholesterol concentration (mg/dL) in negative and positive control groups	**54**
7	Mean HDL-C concentration (mg/dL) in negative and positive control groups	**55**
8	Mean LDL-C concentration (mg/dL) in negative and positive control groups	**55**
9	Heart of rat from negative control group showing the normal histological structure of cardiac myocytes (H & E X 400)	**56**
10	Heart of rat from positive control group showing congestion of myocardial blood vessel and intermuscular few inflammatory cells infiltration	**56**
11	Mean ALT activity (IU/L) in negative and positive control groups	**57**

12	Mean AST activity (IU/L) in negative and positive control groups	**57**
13	Liver of rat from negative control group showing the normal histological structure of hepatic lobule (H & E X 400).	**58**
14	Liver of rat from positive control group showing fibroplasia in the portal triad with appearance of newly formed bile ductuoles (H & E X 400)	**58**
15	Mean blood pressure (mmHg) in negative control and positive control groups	**59**
16	Blood pressure chart of normal control group	**59**
17	Blood pressure chart of positive induction group	**60**
18	Mean cholesterol concentrations (mg/dL) in studied groups after induction and after treatment	**61**
19	Mean triglycerides concentration (mg/dL) in studied groups after induction and after treatment	**62**
20	Mean HDL concentration (mg/dL) in studied groups after induction and after treatment	**64**
21	Mean LDL concentration (mg/dL) in studied groups after induction and after treatment	**65**
22	Heart of rat from ethanolic *Nigella sativa* extract group showing intermuscular oedema (H & E X 400)	**65**
23	Heart of rat from aqueous *Nigella sativa* extract group showing intermuscular oedema (H & E X 400).	**66**

24	Heart of rat from ethanolic mulberry leaves extract group showing no histopathological changes (H & E X 400)	**66**
25	Heart of rat from aqueous mulberry leaves extract group showing congestion of myocardial blood vessel (H & E X 400)	**67**
26	Mean glucose concentration (mg/dL) in studied groups after induction and after treatment	**68**
27	Mean insulin concentration (ng/ml) in studied groups after induction and after treatment	**70**
28	Pancreas of rat from ethanolic *Nigella sativa* extract group showing vacuolation of epithelial cells lining some pancreatic acini (H & E X 400)	**71**
29	Pancreas of rat from aqueous *Nigella sativa* extract group showing no histopathological changes (H & E X 400)	**71**
30	Pancreas of rat from ethanolic mulberry leaves extract group showing no histopathological changes (H & E X 400)	**72**
31	Pancreas of rat from aqueous mulberry leaves extract group showing no histopathological change (H & E X 400)	**72**
32	Mean ALT activity (IU/L) in studied groups after induction and after treatment	**74**
33	Mean AST activity (IU/L) in studied groups after induction and after treatment	**75**
34	Liver of rat from ethanolic *Nigella Sativa* extract treated group showing congestion of central vein and steatosis of some hepatocytes (H & E X 400)	**76**
35	Liver of rat from aqueous Nigella Sativa extract treated group showing steatosis of hepatocytes (H & E X 400)	**76**

36	Liver of rat from ethanolic mulberry leaves extract group showing congestion of hepatic sinusoids and cytoplasmic vacuolization of some hepatocytes	**77**
37	Liver of rat from aqueous mulberry leaves extract group showing steatosis of some hepatocytes (H & E X 400)	**77**
38	Mean blood pressure (mmHg) in studied groups after induction and after treatment	**79**
39	Blood pressure chart of ethanolic Nigella Sativa extract treated group	**79**
40	Blood pressure chart of aqueous *Nigella Sativa* extract treated group	**80**
41	Blood pressure chart of ethanolic mulberry leaves leaves extract treated group	**80**
42	Blood pressure chart of aqueous mulberry leaves extract treated group	**81**