



000000

تم رفع هذه الرسالة بواسطة / سنوي محمود عقل

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

مسئولية عن محتوى هذه الرسالة.

ملاحظات:





Biochemistry Department

Impact of gut microbiota on the epigenetic regulation of Nonalcoholic Steatohepatitis in animal model

A Thesis submitted for the degree of Doctor of Philosophy in Science in
Biochemistry

by

Shaimaa Hamady Gadallah Selim

Assistant lecturer, Biochemistry Department, Faculty of Science
Ain Shams University, M.S.C., 2018

To

Biochemistry Department, Faculty of Science - Ain Shams University

Under the supervision of

Prof. Dr. Hala Mostafa Ghanem

Professor of Biochemistry

Biochemistry Department

Faculty of Science, Ain Shams University

Prof. Dr. Sanaa Eissa Mohamed

Professor of Medical Biochemistry and

Molecular Biology

Faculty of medicine, Ain Shams University

Ass. Prof. Dr. Emad Khairy Ibrahim

Assistant Professor of Biochemistry

Biochemistry Department

Faculty of Science, Ain Shams University

Ass. Prof. Dr. Marwa Matboli Sayed

Assistant Professor of Medical Biochemistry

and Molecular Biology

Faculty of medicine, Ain Shams University

Biochemistry Department

Faculty of Science

Ain Shams University

2022



Ain-Shams University
Faculty of Science
Biochemistry Department

Approval sheet

Impact of gut microbiota on the epigenetic regulation of Nonalcoholic Steatohepatitis in animal model

A Thesis submitted for the degree of Doctor of Philosophy in Science in Biochemistry

Submitted by

Shaimaa Hamady Gadallah Selim

(M.S.C in Biochemistry, 2018)

Supervisors:

- | | |
|-------------------------------------|--|
| -Prof. Dr. Hala Mostafa Ghanem | Professor of Biochemistry, Faculty of Science, Ain Shams University |
| -Prof. Dr. Sanaa Eissa Mohamed | Professor of Medical Biochemistry and Molecular Biology, Faculty of medicine, Ain Shams University |
| -Ass. Prof. Dr. Emad Khairy Ibrahim | Assistant professor of Biochemistry, Faculty of Science, Ain Shams University |
| -Ass. Prof. Dr. Marwa Matboli Sayed | Assistant Professor of Medical Biochemistry and Molecular Biology, Faculty of medicine, Ain Shams University |

Examiners committee:

- | | |
|--------------------------------|--|
| -Prof. Dr. Nadia Mohamed Hamdy | Professor of Biochemistry. The Head of Biochemistry Department, Faculty of Pharmacy, Ain Shams University. |
| -Prof. Dr. Sameh Saad Ali | Professor of Biophysics. The Head of Tumor Biology Program at Children's Cancer Hospital Egypt 57357 |
| -Prof. Dr. Hala Mostafa Ghanem | Professor of Biochemistry, Faculty of Science, Ain Shams University |
| -Prof. Dr. Sanaa Eissa Mohamed | Professor of Medical Biochemistry and Molecular Biology, Faculty of medicine, Ain Shams University |

قَالُوا سُبْحَانَكَ

لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ



Declaration

I declare that this thesis is my own work and has not been submitted for a degree at this or any other university.

Shaimaa Hamady Gadallah

Dedication

*To my mother, my father,
my brothers and my close
friends for their love,
encouragement, help and
prayers that made studies
possible and to them I owe
everything.*

Shaimaa Hamady Gadallah

Acknowledgments

First and foremost, I feel always indebted to Allah the Most Beneficent and Merciful.

*No words could express my sincere appreciation and deepest thanks to **Prof. Dr. Hala Mostafa Ghanem**, Professor of Biochemistry, Faculty of Science, Ain Shams University, for her endless help, close supervision, valuable suggestions and for her sincere efforts and fruitful encouragement.*

*I am deeply indebted to **Prof. Dr. Sanaa Eissa Mohamed**, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Ain Shams University, for her motherly attitude, meticulous supervision, kind guidance, valuable instructions, and generous help.*

*I wish to express my thanks to **Dr. Emad Khairy Ibrahim**, Assistant Professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University, for his generous help, valuable instructions, marvelous and tireless guidance, and profound revision of the manuscript.*

*Really, I can hardly find the words to express my gratitude to my esteemed mentor **Dr. Marwa Matboli Sayed**, Assistant Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Ain Shams University, for her invaluable assistance, active participation, creative thinking, valuable suggestions and tremendous concern and care.*

*I would like to express my thanks to **Prof. Dr. Amany Helmy Hasanin**, Professor of Clinical Pharmacology, Faculty of Medicine, Ain Shams University, for her generous help in designing the animal model and drug administration protocol.*

*Also, I would like to thank **Prof. Dr. Manal El Mahdy**, professor of Pathology, Faculty of Medicine, Ain Shams University, for her assistance in the histopathological and immunohistochemistry studies.*

*My deep thanks and regards to the **Medical Biochemistry Department, Faculty of Medicine, Ain Shams University**, for its kind cooperation and help during the performance of my thesis.*

Last but not least, I would like to thank all my friends and colleagues for their sincere help and encouragement during this study.

 ***Shaimaa Hamady Gadallah***

List of Contents

Title	page no.
List of abbreviations	I
List of Tables	VI
List of Figures	VII
Abstract.....	1
Introduction	2
Aim of the work	5
Chapter I: Review of Literature	6
Nonalcoholic fatty liver (NAFL).....	7
Nonalcoholic steatohepatitis (NASH)	8
Mechanism of hepatic steatosis and lipotoxicity	12
Mechanism of hepatic inflammation and injury	14
Mechanism of hepatic cell proliferation/differentiation and fibrosis.....	17
Contributing factors underlying predisposition to NASH.....	20
• Diet composition and NASH pathogenesis	20
• Gut microbiota and NASH pathogenesis.....	22
• Microbiota-focused strategies for NASH treatment	27
• Hippo signaling pathway and NASH pathogenesis	31
• The Hippo pathway components and regulators.....	31
• Hippo signaling pathway and liver metabolism	38
• Hippo signaling pathway and liver steatosis, inflammation, and fibrosis ...	40
• Therapeutically targeting the Hippo pathway	43
• Genetic/epigenetic factors and NASH pathogenesis.....	44
• Genetics and NASH	44
• Epigenetics and NASH	46

1) MicroRNAs (miRNAs).....	46
-miRNAs and NASH	48
2) Long non-coding RNAs (lncRNAs)	49
-lncRNAs and NASH	50
• Co-regulatory network between mRNAs, miRNAs and lncRNAs	51
• Bioinformatics and mRNAs-miRNAs-lncRNAs network	53
a) Gene Expression Omnibus (GEO) database.....	53
b) miRWalk databases	54
c) Functional annotation and enrichment analysis databases	54
d) STRING database	55
Chapter II: Materials and Methods.....	56
1. In silico studies:.....	56
a) Retrieval of mRNA-biomarkers:	56
b) Retrieval of upstream miRNA-biomarker:	61
c) Retrieval of upstream lncRNA-biomarker:.....	64
2. Experimental studies:.....	66
2.1. Chemicals and drugs:.....	66
2.2. Experimental animals and design:	67
2.3. Blood sampling and liver tissue collection:	68
2.4. Body weight gain and relative liver weight:	69
2.5. Biochemical studies:.....	70
(A) Serum Studies:	70
(B) Tissue Studies:	78
2.6. Molecular assays:.....	87
I. Extraction of total RNA (mRNA, miRNA and lncRNA):	87
II. Synthesis of cDNA:	91
III. Quantitative Real Time Polymerase Chain Reaction (qPCR):.....	94
2.7. Histopathological and immunohistochemical studies:.....	99

I. Hematoxylin-eosin (HE) and Masson's Trichrome staining:	99
II. Immunostaining of alpha-smooth-muscle actin (α -SMA) and large tumor suppressor homologs 1/2 (LATS1/2):.....	100
2.8.Statistical analysis:	101
Chapter III: Results	103
1. The effect of multi-strain probiotic "Flora 20–14 Ultra Strength" and prebiotic "Greena" on body weight and relative liver weight:.....	103
2. The effect of multi-strain probiotic "Flora 20–14 Ultra Strength" and prebiotic "Greena" on liver function tests:.....	107
3. The effect of multi-strain probiotic "Flora 20–14 Ultra Strength" and prebiotic "Greena" on lipid profile tests:	113
4. The effect of multi-strain probiotic "Flora 20–14 Ultra Strength" and prebiotic "Greena" on hepatic IL-6 and TGF- β 1:	116
5. The effect of multi-strain probiotic "Flora 20–14 Ultra Strength" and prebiotic "Greena" on the status of constructed RNA-based regulatory panel:.....	119
6. The effect of multi-strain probiotic "Flora 20–14 Ultra Strength" and prebiotic "Greena" on protein expression of α -SMA and LATS1/2:.....	130
7. Histopathological findings:	138
Chapter IV: Discussion.....	145
Summary	163
References.....	168
Arabic summary	
Arabic abstract	

List of abbreviations

<i>Abbr.</i>	<i>Description</i>
4-AAP	4-Aminoantipyrine
AGO	Argonaute
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMOT	Angiomotin
AMOTL	Angiomotin ligand
AMP	Adenosine monophosphate
AMP	2-amino-2-methyl-1-propanol
AMPK	Adenosine monophosphate-activated kinase
APTR	Alu-Mediated CDKN1A/P21 Transcriptional Regulator
AST	Aspartate aminotransferase
AT	Adipose tissue
ATGL	Adipose TG lipase
cDNA	Complementary DNA
CDS	Coding site
CE	Cholesterol esterase
CFU	Colony-forming units
CK1δ/ε	Casein kinase 1 delta and epsilon
CO	Cholesterol oxidase
Ct	Threshold cycle
DAB	3,3'-Diaminobenzidine
DAG	Diacylglycerol
DAMPs	Danger-associated molecular patterns
DAMPs	Damage-associated molecular patterns
DEGs	Differentially expressed genes
DNL	De novo lipogenesis

DPD	3,5-dichloroaniline
DSBmT	N, N-bis (4-sulfo-butyl)-m-toluidine-disodium
ECM	Extracellular matrix
ER	Endoplasmic reticulum
EtOH	Ethanol
FDA	Food and Drug Administration
FFAs	Free fatty acids
FIAF	Fasting-induced adipocyte factor
FLRL6	Fatty liver related lncRNA
FOS	Fructooligosaccharide
GEO	Gene Expression Omnibus
γ-GT	γ -glutamyl transferase
GK	Glycerokinase
GO	Gene Ontology
GPO	Glycerol-3-phosphate oxidase
GWAS	Genome-Wide Association Study
H&E)	Hematoxylin eosin
HCC	Hepatocellular carcinoma
HDL-C	High-density lipoprotein-cholesterol
HFD	High-fat diet
HPCs	Hepatic progenitor cells
H-score	Histoscore
HSCs	Hepatic stellate cells
HSHF	High sucrose, high fat
HSL	Hormone-sensitive lipase
IFCC	International Federation of Clinical Chemistry
Ihh	Indian hedgehog
IL-6	Interleukin 6
IR	Insulin resistance
JCAD	Junctional protein associated with coronary artery disease
JNK	c-jun N-terminal kinase

KEGG	Kyoto Encyclopedia of Genes and Genomes
KLF6	Kruppel-like factor 6
LATS1	Large tumor suppressor 1
LATS2	Large tumor suppressor 2
LDH	Lactate dehydrogenase
LDL-C	Low-density lipoprotein-cholesterol
lncRNAs	Long noncoding RNAs
LPL	Lipoprotein lipase
LPS	Lipopolysaccharides
MALAT-1	Metastasis Associated Lung Adenocarcinoma Transcript 1
MAMPS	Metabolism associated molecular patterns
MAP4K4	Mitogen-activated protein kinase kinase kinase kinase 4
MBOAT7	Membrane-bound O-acyltransferase domain-containing 7 gene
MCD	Methionine and choline deficient
MDH	Malate dehydrogenase
Mer	Merlin
MeSH	Medical subject headings
MG	Monoglyceride
MGL	Monoglyceride lipase
miRNAs	MicroRNAs
MOB1A	MOB kinase activator 1A
MOB1B	MOB kinase activator 1B
MRE	miRNA Response Elements
MST1	Mammalian sterile20-like kinase 1
MST2	Mammalian sterile20-like kinase 2
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NCBI	National Center for Biotechnology Information
ncRNAs	Non-coding RNAs
NEAT1	Nuclear Enriched Abundant Transcript 1

NF2	Neurofibromin 2
NF-κB	Nuclear factor kappa B
OGT	O-GlcNAc transferase
PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pNP	p-nitrophenol
PNPLA3	Patatin-like phospholipase domain-containing 3
pNPP	p-nitro-phenyl phosphate
PPARα	Peroxisome proliferator-activated receptor alpha
PPI	Protein-protein interaction
PVT1	Plasmacytoma Variant Translocation 1
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RISC	RNA-induced silencing complex
ROS	Reactive oxygen species
RQ	Relative Quantitation
SAV1	Salvador family WW domain-containing protein 1
SCFAs	Short-chain FAs
SNPs	Single nucleotide polymorphisms
SRD5A3-AS1	SRD5A3 antisense RNA 1
SREBP-1c	Sterol regulatory element-binding protein 1c
SREBPc	Mature cleaved nuclear SREBPs
SREBPs	Sterol regulatory element-binding proteins
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TAZ	Transcriptional coactivator with PDZ-binding motif
TC	Total Cholesterol
TEAD	TEA domain transcriptional enhancer factor
TFs	Transcription factors
TG	Triglyceride
TGF-β1	Transforming growth factor-beta 1
TLRs	Toll-like receptors