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ACKNOWLEDGEMENTS

"If you want the cooperation of humans around you, you must make them feel they are important- and you do that by being genuine and humble"

Nelson Mandela

"A tree that wants to touch the sky must extend its roots into the earth. The more it wants to rise upwards, the more it has to grow downwards. So, to rise in life, we must be down to earth, humble & grateful"

A.P.J. Abdul Kalam

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"Nine-tenths of education is encouragement"

Anatole France

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"In time of test, family is best"

Burmese Proverb

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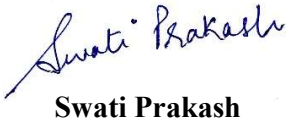
Date: 21st December 2020

Place: Varanasi



Swati Prakash

CERTIFICATE

It is certified that the work contained in the thesis titled ***"Protective role of Sitagliptin in the Treatment of Obesity and associated Metabolic Complications in Animal model of High-fat diet-induced Obesity"*** by ***"Swati Prakash"*** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive, Candidacy and SOTA for the award of Ph.D. degree.

Prof. Sanjay Singh
(Supervisor)

Dr. Vinod Tiwari
(Co-supervisor)

DECLARATION BY THE CANDIDATE

I, ***"Swati Prakash"***, certify that the work embodied in this thesis is my own bonafide work and carried out by me under the supervision of ***"Prof.Sanjay Singh"*** *and* ***"Dr, Vinod Tiwari"*** from ***"July 2014"to "August2020"***, at the **"Department of Pharmaceutical Engineering & Technology"**, Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, *etc*., reported in journals, books, magazines, reports dissertations, theses, *etc*., or available at websites and have not included them in this thesis and have not cited as my own work.

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CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of my knowledge.

Prof. Sanjay Singh
(Supervisor)

Dr. Vinod Tiwari
(Co-Supervisor)

30/12/20

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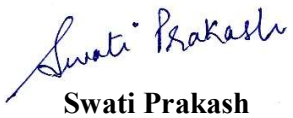

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Dedicated

To

My

FAMILY,

MENTORS,

ALL MY WELL WISHERS

&

DHRUV

List of Abbreviations and Symbols

Absorbance at 260 and 280 nm	- $A_{260/280}$
Alpha	- α
And	- &
Beta	- β
Calcium	- Ca^{2+}
Celsius	- C
Centimeter	- cm
Decilitre	- dL
Degree	- ^{0}
Difference in the values of cycle threshold	- ΔCt
Equal to	- =
Gama	- γ
Gram	- g
Greater than or equal to	- ≥
Hour	- hr
Intraperitoneal	- i.p.
Kilodalton	- KDa
Microgram	- µg
Microliter	- µL
Micrometer	- µm
Milli molar	- mM
Milliampere	- mA
Milli-international units	- mIU
Milliliter	- mL
Millimeter	- mm
Millimoles per litre	- mmol
Minutes	- min
Molar	- M
Nanogram	- ng
Nanometer	- nm
Percentage	- %
Plus minus	- ±
Potential of hydrogen	- pH

Relative centrifugal force	- g
Revolutions per minute	- rpm
Seconds	- sec
Unit	- U
Versus	- vs
Volts	- V
Volume per volume	- v/v
3,3′-Diaminobenzidine	- DAB
5’ adenosine monophosphate-activated protein kinase	- AMPK
60% high-fat diet and 20% fructose water	- HFFW
Acetonitrile	- ACN
Acetyl Co-A carboxylase	- ACC
Adenosine monophosphate	- AMP
Adenosine triphosphate	- ATP
Ammonium Persulfate	- APS
Area under the curve of blood glucose	- AUG
Bovine serum albumin	- BSA
Carnitine palmitoyltransferase-1A	- CPT-1A
Cell Signaling Technology	- CST
c-Jun N-terminal protein kinases	- JNK
Complementary DNA	- cDNA
Concentration	- conc.
Control group	- C
C-reactive protein	- CRP
Deoxynucleoside triphosphate	- dNTP
Deoxyribonucleic acid	- DNA
Diethyl polycarbonate	- DEPC
Dipeptidyl peptidase- IV enzyme	- DPP-IV
Distill water	- DW
Epididymal white adipose tissue	- eWAT
Ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetra acetic acid)	- EGTA
Ethylenediaminetetraacetic acid	- EDTA
Fasting blood glucose	- FBG
Fasting serum insulin	- FSI
Fatty acid synthase	- FASN
Food and Drug Administration	- US-FDA
Genetically induced diabetic mice	- db/db

Genetically induced diabetic mice	- ob/ob
Glucagon insulinotropic peptide	- GIP
Glucagon-like peptide-1	- GLP-1
Glucose tranporter-4	- GLUT-4
Glutathione	- GSH
High density lipoprotein-cholesterol	- HDL-C
Homeostasis model of insulin-resistance	- HOMA-IR
Horseradish peroxidase	- HRP
Hydrocholric acid	- HCl
Inhibitor of nuclear factor kappa-B kinase subunit beta	- IKK-β
Insulin receptor substrate	- IRS-1
Insulin tolerance test	- ITT
Intercapsular brown adipose tissue	- iBAT
Low density lipoprotein-cholesterol	- LDL-C
Magnesium Chloride	- $MgCl_2$
Malondialdehdye	- MDA
Mitochondrial transcription factor A	- TFAM
Mixture	- mix
Monocyte chemoattractant protein-1	- MCP-1
Nicotinamide adenine dinucleotide phosphate	- NADPH
Nitrocellulose membrane	- NC
Nuclear respiratory factor-1	- NRF-1
Nuclease free water	- NFW
One-way Analysis of variance	- ANOVA
Oral Glucose tolerance test	- OGTT
Peroxisome-proliferator-activated receptor gamma co-activator- 1α	- PGC-1α
Peroxisome-proliferator-activated receptor α	- PPARα
Phenylmethylsulfonyl fluoride	- PMSF
Potassium Chloride	- KCl
Potassium dihydrogen phosphate	- KH_2PO_4
Primer melting tempertaure	- Tm
Protein kinase R	- PKR
Radioimmunoprecipitation assay	- RIPA
Reverse-transcriptase Polymerase chain reaction	- RT-PCR
Revolutions per minute	- rpm
Ribonucleic acid	- RNA
Room temperature	- RT

Sisco-Research Laboratories	- SRL
Sodium Chloride	- NaCl
Sodium dodecyl sulphate	- SDS
Standard error of Mean	- SEM
Stromal vascular fraction	- SVF
Superoxide dismutase	- SOD
Tetramethylethylenediamine	- TEMED
Total cholesterol	- TC
Treatment with Metformin 100mg/kg group	- MET
Treatment with Sitagliptin 20mg/kg group	- MS+SGN20
Treatment with Sitagliptin 30mg/kg group	- MS+SGN30
Treatment with Sitagliptin10mg/kg group	- MS+SGN10
Trichloroacetic acid	- TCA
Tris-buffer saline	- TBS
Tris-buffered saline	- TBST
Type-2 Diabetes Mellitus	- DM
Uncoupling protein-1	- UCP-1
Very low-density lipoprotein-cholesterol	- VLDL-C
World Health Organization	- WHO

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PREFACE

With the changes in the global dietary system together with the increased sedentary lifestyle, the global obesity prevalence has nearly tripled since 1975, and it continues to grow at a pandemic rate. Accumulating evidence suggests that obesity is associated with the increased risk of numerous metabolic complications, which is assumed due to the changes in the adipose tissues, leading to premature disability and increased mortality. The remodeling of the white adipose tissues due to excessive fat accumulation induces chronic low-grade inflammation compromising its endocrine function, affecting the release of adipokines along with increased oxidative stress. This shifts the lipid deposition to the ectopic sites inducing metabolic syndrome. Furthermore, fat-enriched diets markedly alter the hepatic gene expression patterns of several structural and metabolic proteins, leading to the development of the fatty liver. Besides, obesity also induces oxidative stress, mitochondrial dysfunction, and affects the thermogenesis activity of the brown adipose tissue. Since, the activity of AMPK, a metabolic sensor involved in energy regulation, is diminished in obesity and fatty liver. Phosphorylation of AMPK and its downstream pathway contributes a significant role in the treatment of obesity, fatty liver, and associated metabolic complications. There is a close association between obesity and the development of insulin resistance, where the occurrence of type-2 diabetes mellitus is common in obesity. In this regard, we hypothesized, the re-purposing of the US-FDA approved oral hypoglycemic drug sitagliptin for the management of obesity and associated metabolic complications in the animal models of high-fat and fructose water diet-induced obesity.

The entire thesis is divided into seven chapters. **Chapter one** includes the introduction to obesity and its pathophysiology, brief discussion related to AMPK, sitagliptin, and the basis of the present study. **Chapter two** deals with an overview of obesity, its epidemiology, complications, and management. Along with this, it lays emphasis on the dysfunctions induced in the adipose tissues in obesity and the role of AMPK in the regulation of obesity. It presents a detailed profile of sitagliptin and discusses the reported clinical observations of sitagliptin on the metabolic syndrome parameters. **Chapter three** illustrates the proposed objective of the study in the high-fat fructose water diet-induced animal model of obesity. **Chapter four** deals with the experimental designs, materials used, and the methods involved in the evaluation of the metabolic syndrome parameters. It discusses the western blotting and the reverse transcriptase-polymerase chain reaction protocols for the evaluation of the protein and gene expressions, respectively. **Chapter five** illustrates the results of the studies performed in previous chapter four. **Chapter six** includes a detailed discussion about the results of the study in reference to the earlier findings. **Chapter seven** concludes the study of the present research work. In the first study, by using the high-fat (60%) fructose water (20%) diet-induced obesity and metabolic syndrome mice model, we observed weight reduction potential with a higher dose of sitagliptin. It improved the metabolic syndrome parameters and improved serum adipokine levels. In the second study in the obese mice model, we found that sitagliptin ameliorated the fatty liver by up-regulating the hepatic adiponectin expression and reduced inflammation in the white adipose tissues. In the third study in the metabolically compromised obese mice, sitagliptin reduced the oxidative stress and improved the thermogenesis and mitochondrial biogenesis markers in the adipose tissues. Thus, our findings suggest the

experimental basis for the treatment of obesity and related metabolic complications with sitagliptin through the activation of AMPK and its downstream targets.