بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / مني مغربي أحمد

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

Ain Shams University
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Autophagy and Rapamycin in Preventing Experimental Diabetes Mellitus Complications

A Thesis

Submitted for the Partial Fulfillment of Philosophy Degree in Pharmaceutical Sciences (Biochemistry)

By

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2022

أعوذ بالله من الشيطان الرجيم

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿فتعالى الله الملك الحق ولا تعجل بالقرآن من قبل

أن يقضى إليك وحيه وقل رب زدني علما﴾

سورة طه ١١٤

Acknowledgment

First of all I thank "**Allah**" for granting me the power to accomplish this work.

I would like to express my deepest thanks to ***Prof. Dr. Hala Osman El- Mesallamy*****, Professor of Biochemistry, Faculty of Pharmacy, Ain Shams University and Dean of Faculty of Pharmacy, Sinai University,** for her valuable scientific supervision, constructive advice and continuous guidance throughout the work.

I would also like to thank ***Prof. Dr. Ahmed Mohamed Mansour*****, Professor of Pharmacology, Faculty of Pharmacy (Boys), AL-Azhar University,** for his active participation and great support during the study.

My deepest gratitude and appreciation are expressed to ***Associate Prof. Nesreen Nabil Omar*****, Acting Head of Biochemistry Department, Faculty of Pharmacy, MTI University,** for her keen, continuous, enthusiastic support patient guidance, and enlightening advice throughout the whole work.

I owe my deepest sincere gratitude to ***Dr. Sherihan Galal Abdel Hamid*****, Lecturer of Biochemistry, Faculty of Pharmacy, Ain Shams University,** for her faithful encouragement, invaluable suggestion and advice throughout the work.

Finally, my deepest everlasting thanks and appreciation are for ***my family*****,** for their continuous support and encouragement during the whole thesis tiring period.

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List of Abbreviations

Abbreviation	Definition
ABCB1	Adenosine triphosphate binding cassette subfamily b member 1
AGEs	Advanced glycation end products
Akt	protein kinase B
Ambra1	Autophagy and beclin 1 regulator 1
AMPK	Adenosine monophosphate-activated protein kinase
Atg	Autophagy related gene or protein
BHB	β -hydroxybutyrate
CMA	Chaperone-mediated autophagy
CRP	C-reactive protein
DFCP1	Zinc finger FYVE (Fab 1, yotB, vesicle transport protein and early endosome antigen 1) domain-containing protein 1
DM	Diabetes mellitus
DN	Diabetic nephropathy
DR	Diabetic retinopathy
ER	Endoplasmic reticulum
ESRD	End-stage renal disease
FBG	Fasting blood glucose
FDA	The Food and Drug Administration
FKBP-12	Immunophilin 12- kda FK506- binding protein
F-STZ	Fasting-Streptozotocin
GBM	Glomerular basement membrane
GDM	Gestational diabetes mellitus
GFR	Glomerular filtration rate
GSH	Reduced glutathione
H&E	Hematoxylin and eosin stain
HDL-C	High density lipoprotein cholesterol
Hsc70	Heat-shock cognate 70
IR	Insulin resistance
LAM	Lymphangioleiomyomatosis
LC3	Microtubule-associated protein 1A/1B-light chain 3
LDL-C	Low density lipoprotein cholesterol
LKB1	Liver kinase B1
LPL	lipoprotein lipase
MDA	Malondialdehyde
MDR1	Multidrug resistance protein 1

Abbreviation	Definition
MS	Mesangial sclerosis
mTOR	Mammalian target of Rapamycin
mTORC1	Mammalian target of Rapamycin complex 1
mTORC2	Mammalian target of Rapamycin complex 2
NADPH	Nicotinamide adenine dinucleotide phosphate
PAG	Polyacrylamide gel electrophoresis
PI3K	Phosphatidylinositol 3-kinases
PI3P	phosphatidylinositol 3-phosphate
PKC	Protein kinase C
POD	Peroxidase
PTEN	Phosphatase and tensin homolog
PVDF	Polyvinylidenene difluoride
Ras	Rat sarcoma virus
RIPA	Radioimmunoprecipitation assay
ROS	Reactive oxygen species
R-STZ	Rapamycin-treated Streptozotocin
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAG	Triacyglycerol
TC	Total cholesterol
TEM	Transmission electron microscope
TSC	Tuberous sclerosis complex
ULK1/2	Unc-51 like autophagy activating kinase
UNC-51	Serine/threonine-protein kinase
Vps34	Phosphatidylinositol 3-kinase
WB	Western blot
WIPI2	β-propeller repeat domain phosphoinositide-interacting protein 2

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1. Introduction and Aim of the Work

Diabetes mellitus (DM) is considered a global emergency. Around 537 million people worldwide suffer from DM, and1.6 million die annually secondary to diabetes ***(IDF, 2021)***. It is predicted that 643 million people to suffer from DM by 2030 and to 783 by 2045. ***(WHO, 2020; Patel et al., 2022)***.

Many pathological factors are involved in the prognosis of DM. However, pancreatic β cell dysfunction is considered the core of DM and its complications ***(American Diabetes Association, 2014; Antonetti et al., 2021)***.

Maintenance of pancreatic β cells could be achieved through two strategies. First, increasing its reproductive capacity. However, the potential risk of cancer emergence may accompany this strategy. Second, enhancing the defense power of the β cells against destructive matters by inducing autophagy. Nevertheless, the subsequent risk of programmed cell death might also arise. Consequently, an optimal induction of autophagy is the key to gain the benefits of this vital biological process ***(Stützer et al., 2012; Vetere et al., 2014; Eshraghi et al., 2022)***. We aimed in this study to prolong the life span of β cells through induction of

autophagy in an appropriate way. Autophagy has been demonstrated to have a beneficial effect on DM-induced nephropathy ***(Khodir et al., 2020)***.

Rapamycin, an immunosuppressant mammalian target of Rapamycin (mTOR) inhibitor drug was shown to stimulate β-cell autophagy, but its effects on preventing or ameliorating the diabetic nephropathy (DN) is unclear, an effect worth to be studied.

Research on fasting is gaining attraction based on recent studies that show its role in many adaptive cellular responses such as the reduction of oxidative damage and inflammation ***(Visioli et al., 2022)***. Fasting forces healthy cells to enter a slow division and highly protected mode that protects them against oxidative stress which is considered a key factor in developing pancreatic insufficiency ***(Nencioni et al., 2018)***. As fasting is now an attractive protective strategy, its effect will be compared to Rapamycin effects on pancreatic and renal cells.

Induction of autophagy in pancreatic β cells is of an appreciated importance, as it results in indirect activation of CAT biosynthesis that is normally expressed in low level in

the pancreatic β cells ***(Lenzen et al., 1996; Pearson et al., 2021)***.

Accordingly, the ultimate aim of this study was to:

1) Explore the real outcome of autophagy in developing or preventing DM complications, in particular DN.
2) Estimate the prophylactic importance of Rapamycin, as a standard inducer for the autophagy, in preventing progression of DN.
3) Explore the physiological importance of fasting towards DM and pancreatic β cells.
4) Compare the effect of Rapamycin/ fasting in enhancing or worsening DM and its complications.