SCREENING OF NATURAL EPIGENETIC MODIFIERS FOR MANAGING GLYCEMIC MEMORY AND DIABETIC NEPHROPATHY

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Biotechnology

By

Kriti Kushwaha

Registration Number: 11814424

Supervised by

Dr. Jeena Gupta

Associate Professor

Department of Biochemistry

School of Bioengineering and Biosciences

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2023

DECLARATION

I, hereby declared that the presented work in the thesis entitled "Screening of natural epigenetic modifiers for managing glycemic memory and diabetic nephropathy" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr. Jeena Gupta, working as Assistant professor, in the Biochemistry department of School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Name of the scholar: Kriti Kushwaha

Registration No.: 11814424

Department/school: Biotechnology (School of Bioengineering and Biosciences)

Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Screening of natural epigenetic modifiers for managing glycemic memory and diabetic nephropathy" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Biochemistry department of School of Bioengineering and Biosciences, is a research work carried out by Kriti Kushwaha, 11814424, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)

Name of supervisor: Dr. Jeena Gupta

Designation: Associate Professor

Department/school: Biochemistry, School of Bioengineering and Biosciences

University: Lovely Professional University

ABSTRACT

Diabetes is a chronic medical condition that affects millions of people worldwide, and its prevalence is increasing rapidly. It was grossly underappreciated as a worldwide health issue until the last decade. Due to a lack of precise data for monitoring and surveillance, there are significant gaps in efforts to comprehend the burden on a national and global scale, particularly in developing countries. Diabetes mellitus (DM) has now become a global pandemic with the number of patients increasing every year. Diabetes affects more than 29 million individuals in the United States of America and 420 million people all around the globe with a global prevalence of 642 million expected by 2040. This escalating pandemic imposes significant human and financial consequences on individuals, society, and the economy. The growing number of antihyperglycemic medication options for type 2 diabetes, which frequently involves different mechanisms of action and safety profiles, can be difficult for clinicians to manage, and the increasing complexity of diabetes management necessitates a well-informed strategy for diabetes prevention and treatment.

Diabetes is a metabolic and lifestyle disorder with a multitude of factors responsible for its onset. Insulin resistance, the most prevalent underlying abnormality, is caused by an imbalance in energy intake and expenditure, which favors nutrient-storage pathways, evolved to maximize energy consumption while preserving enough substrate supply to the brain. Firstly, white adipose tissue malfunction and circulating metabolites influence tissue communication and insulin signaling. When the energy imbalance is chronic, systems such as inflammatory pathways speed up these abnormalities. Persistent high level of glucose leads to the development of glycemic memory (GM) where the kidney keeps getting worse even after the glucose has returned to normal. Diabetic kidney disease (DKD), also known as diabetic nephropathy, is a common complication of diabetes, and it is influenced by both genetic and environmental factors. DKD kills many people worldwide which prompts us to get a better knowledge of it. Genes related to certain DKD traits are regulated by epigenetic mechanisms.

Sirtuin-1 (SIRT1) has been a new and novel target in metabolic diseases and scientists have been working for the last decade on elucidating its role in diabetic nephropathy (DN). Many drugs are available in the market to treat DM along with nephropathy of which metformin is the most successful one. But even a drug as good as metformin has side effects which led us to fish out phytochemicals that can help in managing GM and DN by modifying the epigenetic axis.

We undertook this present study to screen natural epigenetic modifiers that had structural similarities with existing phytochemicals known for managing GM and DN. For screening, we use ChEMBL and PubChem followed by unraveling SIRT1 activity through site-specific molecular docking using Autodock Vina 1.5.6 and visualizing results on python molecule viewer. Potent molecules were then further analyzed by SwissADME to study Absorption, Distribution, Metabolism, and Excretion (ADME) properties followed by checking their efficacy in, in vitro as well as in vivo models of GM and DN. In silico analysis helps us to screen molecules from a pool of potent phytochemicals where time and resources are an issue.

With the structural similarity searching approach, we found phytochemicals similar to each of our parent molecules: metformin, resveratrol, vanillic acid, esculetin, genistein, and berberine. Similar phytochemicals were then subjected to docking where further screening was done on the basis of binding energy, binding pose, and binding modes. To check the fate of our drugs ADME properties were checked and after all the in-silico studies, we chose Gamma amino butyric acid (GABA) and umbelliferone (UMB) for further analysis. GABA is an inhibitory neurotransmitter in the human central nervous system (CNS) and other mammals. It is made from glutamate-by-glutamate decarboxylase (GAD) and stored in presynaptic vesicles. GABA also improves cell survival and regeneration, resulting in an increase in cell mass. Additionally, because GABA reduces systemic inflammatory and cytokine production, GABA therapy in modulating glucose homeostasis in the management of DM is promising. GABA taken orally is safe for humans. It operates on peripheral GABA receptors but has no effect on CNS functioning because it is not able to pass the blood-brain barrier (BBB). Hence it can be a new therapeutic approach for both DN as well as GM. Plant-derived phenolic coumarins may serve as dietary antioxidants due to their presence in the human diet, particularly in fruits and vegetables. Natural antioxidant UMB (7-hydroxycoumarin) is benzopyrone in nature, and it is found in edible fruits such as golden apples and bitter oranges. Coumarin, the parent compound, has been shown to lower plasma glucose levels.

Both the phytochemicals exhibited remarkable antioxidant potential and the results of the cell viability assay performed using (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay analyzed their influence on cell proliferation of the 3T3L-1 cell line and found that our phytochemicals are non-toxic to normal cells ex-vivo. In vitro GM was established by reversing glucose conditions that caused elevation in ROS production. Upon administering phytochemicals, the oxidative stress caused by the hyperglycemia was ameliorated as observed by monitoring oxidative biomarker lipid peroxidation (LPO), and improving activities of antioxidant enzymes Catalase, Superoxide Dismutase (SOD), and Glutathione-S-Transferase (GST). In vitro results suggest that phytochemicals are good antioxidant compounds and may help in alleviating damage to the cells.

GABA and UMB were further evaluated in vivo in a High-fat-diet induced GM model in Sprague-Dawley rats. The results of in vivo study elucidated that GABA and UMB successfully lowered the increased blood plasma glucose, total cholesterol, serum triglycerides, very-low-density lipoprotein (VLDL), low-density lipoproteins (LDL), Alkaline phosphatase (ALP), Serum Glutamic Pyruvic Transaminase (SGPT), and serum glutamic-oxaloacetic transaminase (SGOT). Histopathology of the renal section showed glomerular hypertrophy in HFD rats but upon treatment with GABA and UMB, reduced kidney damage was observed. Altogether, we conclude that GABA and UMB protect from nephropathy caused due to hyperglycemia and glycemic memory. GABA and UMB also act as anti-hyperlipidemic and nephroprotective agents.

Acknowledgement

वक्रतुण्ड महाकाय सूर्यकोटि समप्रभ |
निर्विघ्नं कुरु मे देव सर्वकार्येषु सर्वदा ||

All glory to the omnipresent for granting me the tenacity and patience to accomplish this research project. I give thanks to God for making things possible for me to overcome adversities. In the moments when I was on an emotional roller coaster, it was the word of Lord **Shri KRISHNA** from **Shrimadbhagwat GITA** "*I have a purpose for your pain, a reason for your struggle, and a reward for your faithfulness*" that kept me going.

I want to acknowledge my debt and convey my heartfelt appreciation and gratitude to my respected supervisor **Dr. Jeena Gupta Bansal**, Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University for her attentive instruction, constructive criticism, vast knowledge, scientific suggestions, altruistic behavior and unwavering support in completing this thesis. It has been a great joy and honor to work under her supervision.

I would like to express my gratitude to **Dr. Navneet Khurana**, Associate Professor, Department of Pharmacology, School of Pharmaceutical Science, LPU, for generously sharing his time and knowledge in performing my *in vivo* investigations and for his hard efforts in completing them successfully.

I would like to express my heartfelt gratitude to **Prof. Neeta Raj Sharma**, Head of the School of Bioengineering and Bioscience, **Dr. Umesh Gautam**, Head of the Department of Biotechnology, **Chirag Chopra**, Head of Laboratory School of Bioengineering and Bioscience, and all other faculty members of the School of Bioengineering and Bioscience for providing me with the facilities to conduct my research and for their ongoing support.

I'd like to take this opportunity to thank **Mr. Ashok Mittal** (Chancellor), **Mrs. Rashmi Mittal** (Pro-Chancellor), **Dr. Preeti Bajaj** (Vice Chancellor), **Dr. Loviraj Gupta** (Executive Dean), and **Dr. Monica Gulati** (Registrar) for allowing me to serve at such a distinguished university.

I'd also like to express my gratitude to our lab technicians, **Mr. Rahul Sharma, Mr. Kuldip, Mr. Sunny, Mr. Rajesh, Ms. Sandeep Kaur, and Mr. Madan**, who helped me at every level of my research.

The encouragement, support, and help of my friends can never be put into words and for that, I would like to thank my peers **Aayushi Velingkar, Jeenu Cherian,** and **Vaishali Dhaka** for always keeping up with me and for being there always.

I'd like to extend my thanks to my senior **Sana Shafi Bhat** for bearing with me and being an inspiration. I'd also like to thank my juniors **Nancy Bhura, Sourbh Garg**, and **Debojyoti Mandal** for their untimely help. A special appreciation to my dear friend and colleague **Rupal Dubey** for her moral support throughout my journey.

To my parents, I owe more than words can ever express. For all I am today and will be tomorrow, it's because of my dear papa, **Mr. Ram Asrey Kushwaha**, and my beautiful maa **Mrs. Kiran Kushwaha.** I can never repay you for your constant willingness to provide anything I needed, and your unending sacrifice has helped me to get where I am today. I will be eternally grateful to God for providing me with parents who are always supportive of my goals and who love me unconditionally. Thank you, maa and papa, for believing in me; you are my role models. I couldn't have bloomed without you.

To my sisters **Reeti Kushwaha** and **Arohi Kushwaha** who mean the world to me, just know that I wouldn't have made it if it weren't for you both, supporting and holding me. Thank you for being you.

I'd also like to thank **Mr. Pranjal Rai** for being a pillar I can always rely on.

Last, but not least, I'd want to thank everyone who, whether directly or indirectly, helped me complete my experimental work and thesis.

Kriti Kushwaha

Date 19 July, 2023

Table of Content

List of Tables

LIST OF FIGURES

Abbreviations

DM	**Diabetes mellitus**
DN	Diabetic nephropathy
SIRT1	Sirtuin-1
DKD	Diabetic kidney disease
ADME	Absorption, distribution, metabolism, and excretion
GABA	Gamma amino butyric acid
UMB	Umbelliferone
CNS	Central nervous system
BBB	Blood-brain barrier
GM	Glycemic memory
LPO	Lipid peroxidation
SOD	Superoxide dismutase
GST	Glutathione s transferase
HFD	High-fat diet
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
ALP	Alkaline phosphatase
VLDL	Very-low-density lipoprotein
LDL	Low-density lipoproteins
WHO	World health organization
EDIC	The Epidemiology of Diabetes Interventions and Complications
DCCT	Diabetes Control and Complications Trial
ESRD	End-stage renal disorder
VSMC	Vascular Smooth Muscle cell
REC	Retinal endothelial cells
ECM	Extracellular matrix
ROS	Reactive oxygen species
AGES	Advanced glycation end products
PKB	Protein kinase B
MIRNA	Micro RNA
PTMS	Post-translational modifications
HKAC	Histone lysine acetylation
DNAME	DNA methylation
HAC	Histone acetylation

HATS	Histone acetyltransferases
HDAC	HITSONE deacetylase
CBP	CREB binding protein
COX-2	Cyclooxygenase-2
TNF-A	Tumor necrosis factor-alpha
MCP	Monocyte chemoattractant protein
RAAS	Renin-Angiotensin aldosterone System
DPP4	Dipeptidyl peptidase IV
SGL	Sodium-glucose transporter
HMG-COA	Hydroxy-methylglutaryl coenzyme A
ACEIS	Angiotensin-converting enzyme inhibitors
ARBS	Angiotensin receptor blockers
TGF-B	Transforming growth factor-β
HSP	Heat shock protein
MAPK	Microtubule-associated protein kinase
GLP-1	Glucagon-like peptide-1
IDF	International diabetes federation
TXNIP	Thioredoxin interacting protein
MTOR	Mammalian target of rapamycin
DAG	Diacylglycerol
VEGF	Vascular endothelial growth factor
PAI	Plasminogen activator inhibitor
NF-KB	Nuclear factor kb
SMAD3	Small mothers against decapentaplegic homolog 3
MAP3K	Mitogen-activated protein kinase kinase kinase
MAPKK	Mitogen-activated protein kinase kinase
JNK	Jun N-terminal kinase
SAPK	Stress-activated protein kinase
ERK5	Extracellular-regulated kinase 5
BMK	Big mitogen-activated protein kinase
AP	Activated protein-1
CTGF	Connective tissue growth factor
TEF	Transcription enhancer factor
MEF	Myosin enhancer factor
IL	Interleukins

RAF	Rapidly accelerated fibrosarcoma
ROCK	Rho-associated protein kinase (ROCK)
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
GAPD	Glyceraldehyde-3-phosphate dehydrogenase
IFN	Interferon
CD	Cluster of differentiation
VCAM	Vascular cellular adhesion molecule
ICAM	Intercellular adhesion molecule
RASAL-1	Ras protein activators like 1
HMTS	Histone lysine methyltransferases
PPAR-Γ	Peroxisome proliferator-activated receptors
STZ	Streptozotocin
GFR	Glomerular filtration rate
UAE	Urinary albumin excretion
HPLC	High-performance-liquid chromatography
DPPH	1,1 diphenyl-2-picrylhydrazyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's modified eagle medium
PBS	Phosphate buffer saline
EDTA	Ethylenediamine tetraacetic acid
BSA	Bovine serum albumin
CDNB	1-chloro-2,4-dinitrobenzene
MDA	Malondialdehyde
TBA	Thiobarbituric acid
NBT	Nitroblue tetrazolium
HRP	Horseradish peroxidase
TMB	Tetra methyl benzidine
ANOVA	Analysis of variance
PMSF	Phenylmethylsulfonyl fluoride
LSB	Low salt buffer
ECL	Enhanced chemiluminescence0p
RRT	Renal replacement therapy
SDS	Sodium dodecyl sulfate

CHAPTER 1

INTRODUCTION

Diabetes

Recent World Health organization (WHO) reports highlight that the number of diabetic cases has nearly doubled since 1980, as it rose from 4.7% to 8.5% in the adult generation (IDF Diabetes Atlas, 2022). The cause of diabetes is major because of both genetics and lifestyle factors (Ripsin et al, 2009). A multitude of lifestyle factors modulates diabetes progression. One of the terms generally associated with diabetes is obesity, which has been found to contribute to nearly 55% of cases of diabetes. Between the 1960s and 2000s, there was an increase in the rate of obesity during childhood that eventually led to an increase in cases of diabetes in adolescents and children (Barlow et al, 2007). Many of the environmental toxins may contribute to the recent increases in the rate of diabetes.

Glycemic memory (GM)

One of the hallmark features of DM is hyperglycemia. Diet and environment have a direct influence on the growing realization that even a transient episode of hyperglycemia produces long-lasting effects that persist after following strict glycemic control. There is evidence of increased micro and macrovascular complications of diabetes with increasing duration of hyperglycemia. These conditions prolong even after glycemic control is achieved. This is indicative of an innate mechanism of "metabolic or glycemic memory". This so-called "*glycemic memory*" is due to some irreversible mitochondrial and vascular changes which produce sustained effects, worsening endogenous vascular and renal systems. GM is also a synonym for GM (Ihnat et al., 2007 and Ceriello et al., 2009) and it is an innate mechanism where diabetic complications continue to worsen even after good glycemic control is achieved (Paneni et al., 2013).

This condition has been reported in clinical trials and in animal models as well. The Epidemiology of Diabetes Interventions and Complications (EDIC) study and Diabetes Control and Complications Trial (DCCT) have been done in these types of studies (The DCCT/EDIC Research Group 2002). The result from DCCT depicted that Type 1 diabetes patients who were under intensive glycemic control had less chance of developing diabetic complications like neuropathy, and nephropathy than those who were under standard therapy. Other trials held for T2DM patients suggested that the benefit of intensive glycemic control was found to be lasted long even after ceasing strict routine: a 'legacy effect' (Chalmers and Cooper 2008). Even strict glycemic control could not overcome the effect of GM and leads to end-stage renal disorder (ESRD) (Villeneuve et al., 2011).

Roy et al., 1990 get the credit for first reporting GM in animal models and cell cultures. GM models have also been mimicked in cell culture that shows an unusual expression of antioxidant, profibrotic and inflammatory genes in Vascular Smooth Muscle Cell (VSMCs) (Reddy et al., 2012), retinal endothelial cells (RECs) (Kowluru 2003; Zhong and Kowluru 2011, 2013 a & b) as well as endothelial cells (Brasacchio et al., 2009) despite normal levels of glucose. In animals also, metabolic models have been successfully achieved with complications like

retinopathy, nephropathy (Kowluru 2003, Zhong and Kowluru 2011, 2013 a & b) and atherosclerosis (Brasacchio et al., 2009).

Although, both *in-vitro* as well as *in-vivo* studies have shown that achieving good glycemic control is important in managing diabetes and reducing the risk of complications. However, even after achieving glycemic control, the effects of previous hyperglycemic exposure on target organs can continue to affect the body. This phenomenon is known as GM which refers to the long-term effects of hyperglycemia on the body's cells and tissues. GM can contribute to the development and progression of diabetes complications. As a result, people with diabetes need to be vigilant about maintaining good glycemic control, even if they have achieved it in the past, to minimize the risk of long-term complications.

Issues related to diabetes

Non-insulin-dependent diabetes mellitus (NIDDM) is known for insulin resistance, and insulin deficiency (Maitra et al, 2005). Interaction between environmental, behavioral, and genetic risk factors is a major cause of T2DM (Chen 2011). T2DM is said to be more vulnerable, and people affected by it can have long and short-term implications that can lead to premature death. Diabetes affects vital organs of the body and complications associated with it are categorized as microvascular (like retinopathy, nephropathy, neuropathy, etc.) and macrovascular (coronary/peripheral artery disease, stroke, etc.) complications (Frank.,2004).

Diabetic Nephropathy

Approximately 20-40% of diabetic patients develop nephropathy (which may also be the side effect of diabetes-curing drugs) which eventually converts to ESRD (Kanwar et al., 2011). Persistent high glucose, high insulin, and proteinuria cause the accumulation of extracellular matrix (ECM) protein, deposition of collagen and laminin, and chronic relentless fibrosis leading to glomerulosclerosis and renal failure (Ban and Twigg, 2008). Factors affecting DN mainly comprise reactive oxygen species (ROS), persistent hyperglycemia, proinflammatory cytokines, advanced glycation end products (AGEs), protein kinase B (PKB), Adenosine monophosphate-activated kinase (AMPK) and thioredoxin interacting protein (Fukami et al., 2008). Diabetes causes anomalies in the expression of proinflammatory genes (Sanchez et al., 2009; Kanwar et al., 2011 and Woroniecka et al., 2011). The conditions get worse as DN progresses which makes the kidney impotent to clear toxins from the body (Li et al, 2004). There are different procedures used to treat DN but the complex mechanism of DN makes the treatment expensive and complicated. The current therapies include the use of medications for blood pressure and diabetes whereas inhibitors of the angiotensin-converting enzyme (ACE) are used at an early stage. Current treatment strategies also include the use of diuretics, renal replacement therapy, and beta-blockers along with prescribed medications, making this even more expensive (Shafi et al., 2012).

Diabetes and Epigenetics

"*Epigenetics*" refers to heritable and also reversible, changes in the DNA methylation, histone modifications, micro RNA (miRNA) levels, and chromatin structure responsible for regulating gene expression without changing the underlying DNA structure. The epigenetic state of an individual's DNA is both inherited and modifiable, such that DNA expression patterns can be passed from parent to offspring, or they can be modified in response to environmental triggers (Whitelaw et al, 2006). Waddington (1942) originally explained 'epigenetics'. The epigenome is not a constant thing and is modulated by different chemical x-factors and environmental factors. (Riancho et al., 2014). Epigenetic modifications are DNA methylation, histone modifications, and RNA silencing (Kelly et al., 2010; Handy et al., 2011; Allis and Jenuwein, 2016). These mechanisms regulate the structure of chromatin to bestow transcription memory; important for installing gene expression patterns during cell divisions even when the initiation signals are absent. The role of epigenetic control lies in cell identity, cellular responses to environmental signals, genomic imprinting, embryogenesis, X-chromosome inactivation, the plasticity of stem cells, and immune cell functions (Kouzarides, 2007 and Jones, 2012). Changes in epigenomic states can have a deep effect on regulating gene expression and the result of this is often associated with diseased conditions (Portella and Esteller, 2010 and Baylin and Jones, 2011). Even after the removal of original stimuli, the long-term persistent epigenetic modifications may contribute to diseases like diabetes and its associated complications. Several environmental factors influence the epigenetic mechanism that causes abnormal gene expression involved in cardiovascular and metabolic disorders (Wang et al., 2012). Many emerging studies have demonstrated that multiple signaling pathways and transcript factor activation are involved in DN progression. Shreds of evidence have shown an unusual relationship between genes and the environment that shows changes in gene expression in some metabolic disorders (Keating and Osta 2015 and Pirola et al., 2010). Mechanisms that are modulated epigenetically like DNA methylation, miRNA, and post-translational modifications (PTMs) can modify gene expression in cells. Histone proteins are involved in PTM like histone lysine acetylation (HKAc), phosphorylation, ubiquitination, and histone lysine methylation (HKMe) (Reddy et al., 2013 and Li et al., 2016) **(Figure 1.1).** Epigenetic mechanisms, including histone modifications, DNA methylation (DNAMe), and non-coding RNAs such as microRNAs, have been found to play important roles in the development and progression of various diseases, including DN. (Miao et al., 2004; Ha, 2011 and Villeneuve et al., 2011).

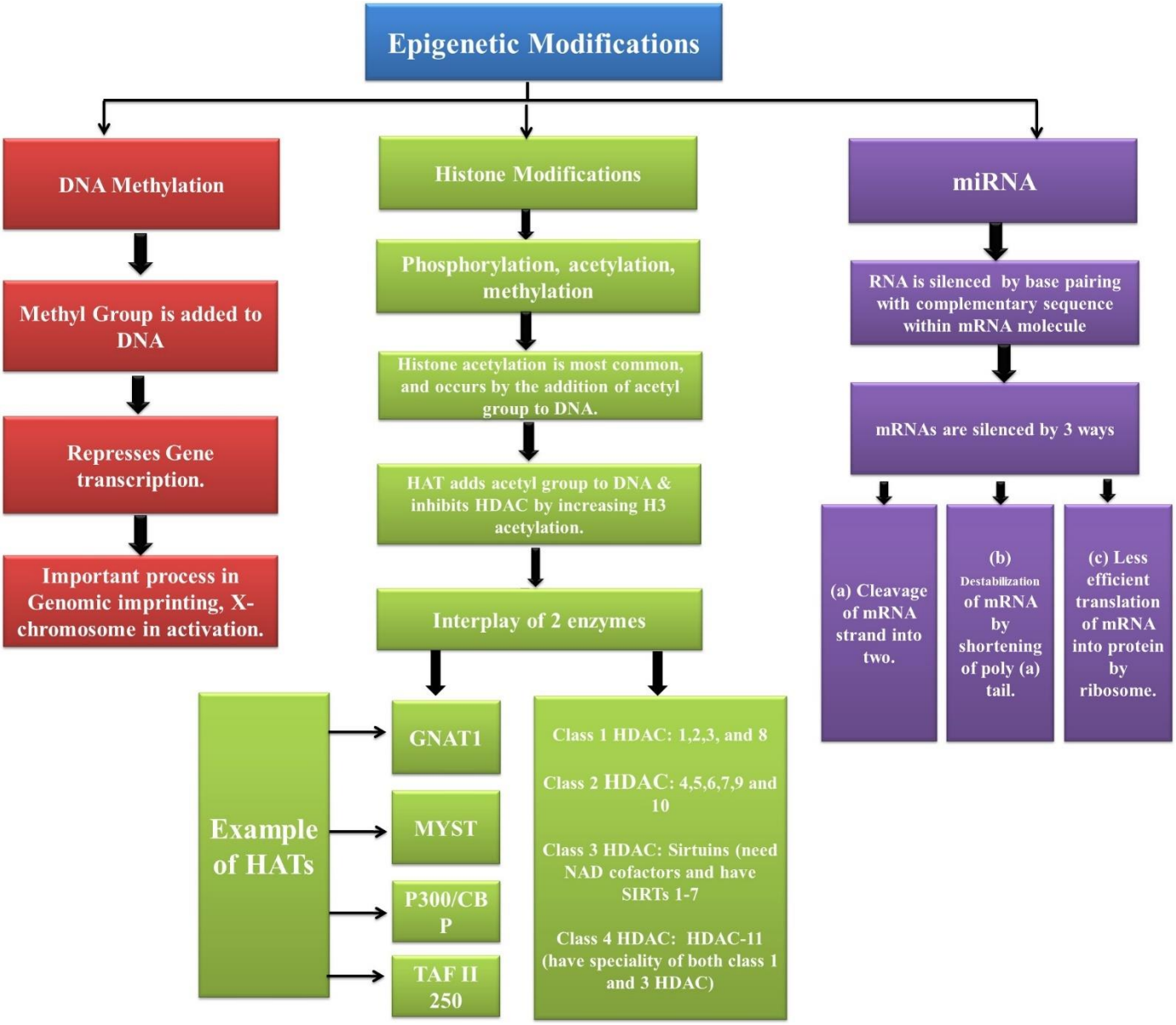


Figure 1.1: Types of epigenetic modifications involved in DN. DNA methylation is shown to be involved in genomic imprinting; phosphorylation, acetylation, and methylation are some of the histone modifications that are modulated by HATs like GNAT1, MYST and HDACs like HDAC1-4; while miRNA silencing is done by base pairing within mRNA molecules.

DNAme converts the nucleotide of DNA, cytosine, into 5-methyl-cytosine. In a nutshell, it makes the fifth base in the genes that are adjacent to the CpG islands present at the DNA promoter region (Esteller, 2008). HAc is one of the major PTMs in which the acetyl group is added to amino-terminal tails of histone to facilitate or repress the transcription of genes. It is achieved by changing the interaction of DNA to that of histones (Brasacchio et al., 2009 and Grace et al., 2012). Non-coding RNA is known to provide both transcription and translation. These can change chromatin structure directly or indirectly with the help of chromatin-modifying complexes to target gene loci. miRNA is present at the 3' untranslated region of mRNA to regulate gene expression by mRNA degradation and translational repression (Bartel, 2004; Guttman and Rinn, 2012 and Kornfeld and Brunning, 2014).

Histone acetylation (HAc) is one of the major epigenetic marks where lysine residues at the N terminus of histones get acetylated which removes positive charges (Waterberg, 2002). Acetylation of histone helps in regulating mitosis, PTM, gene expression, and assembly of the chromosome by changing the structure of chromatin but not changing DNA sequences (Zhang et al., 2015). The HAc level in the cells is regulated by the interplay between two types of histone-modifying enzymes: Histone Acetyl Transferases (HAT) and histone deacetylase (HDAC). A multitude of reports has demonstrated altered HAc patterns under diabetic conditions (Gupta et al., 2012 and Martinez-Jimenez and Sandoval, 2015). The change in the pattern of HAc and recruitment of HAT/HDACs on gene promoters are known to modulate kidney gene expression and increase renal fibrosis under diabetic conditions (Sun et al., 2016).

Histone acetyltransferases (HATs) like CREB binding protein (CBP)/p300 and Steroid receptor co-activator (SRC-1) and HDACs I-IV are used for the catalyzation of HAc (Roth et al., 2001 and Yang and Seto, 2008). HAc marks on H3 and H4 are mostly studied. HKAc (acetylation of lysine residues on N terminal histone chains) helps in gene transcription by making DNA easily accessible for the transcription factors to bind. HKAc neutralizes the positive charge present on the histone proteins and weakens the binding to negatively charged DNA (Lu et al., 2015). H3K9Ac, H4Kac, and H3K14Ac are examples of permissive gene expression (Reddy and Natrajan, 2011). Histone deacetylation occurs when chromatin gets condensed and hence there is repression in gene transcription (Campos and Reinberg, 2009 and Mathiyalagan et al., 2014).

Many reports are there that identify a relationship between HAc and DN progression. A study by Miao et al., 2004 reported that H3 and H4 acetylation at lysine residue numbers 14 and 9 and 8, 5, and 12 respectively were found to be increased at cyclooxygenase-2 (COX-2) and tumor necrosis factor (TNF-α) gene promoters. In another report, it was shown that oxidation of lipids may also increase H3K9/14Ac on monocyte chemoattractant protein (MCP-1) promoter region of genes (Reddy et al., 2009 and Kanwar et al., 2011).

Histone deacetylation is mediated by an enzyme called HDACs (Reddy et al., 2013). HDAC removes an acetyl group from lysine residues. HDACs are categorized into 4 classes. 18 human HDACs have been identified as of now. Class III HDAC comprises of sirtuins (1-7). Class IV HDAC comprises of HDAC 11 (Keppler and archer, 2008). Many reports have been accumulated that tell how HDAC controls DN. In a study on Akita mice, it was shown that HDAC1 levels were decreased in HBZY-1 cells under diabetic conditions leading to an increase in factors associated with inflammation and hence progressing DN (Chen et al., 2014).

Silent mating type information regulation (SIRTs) are very diverse molecules and regulate processes like apoptosis, cell cycle, and insulin secretion. Malfunctioning of SIRTs leads to several metabolic disorders like diabetes, obesity, cancer, and neurological disorders (Seto and Yoshida, 2014).

Therapies and drugs have been in use to prevent epigenetic modifications and oxidative stress, with the potential to ameliorate diabetic nephropathy. Metformin is the only known drug to have successfully ameliorated GM (Tikoo et al., 2016). There are also targeted therapies like Renin-Angiotensin aldosterone System (RAAS) blockers, statins, fibric acid derivatives, and thiazolidinediones (TZDs) that treat diabetic nephropathy.

Evolving therapies include inhibiting sodium-glucose transport. For glycemic control, oral drugs are present that are given to patients before insulin therapy. Sulfonylureas are used for those who have chronic kidney disease. TZDs decrease insulin resistance and are insulin sensitizers. TZDs may increase the chances for the progression of DN by decreasing microalbuminuria (Wang et al., 2010). There are many oral drugs like dipeptidyl peptidase IV (DPP4) inhibitors, meglitinides, and alpha-glucosidase inhibitors whose potential in curing DN is still to be unrevealed. Another target to control blood glucose levels is to decrease proximal tubular glucose reabsorption by selectively blocking the sodium-glucose transporter, (SGL2) by SGL2 inhibitor (Bakris et al., 2009). Diabetic patients have high triglycerides and cholesterol. Hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) is another therapy to target LDL (Haffner, 2003). Many studies have reported a decrease in microalbuminuria with statin use (Fried et al., 2001). The use of RAAS blocking agents helps in decreasing intraglomerular pressure and hyperfiltration which decreases urine protein excretion (Parving *et al.*, 2008). Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) retarded the advancement of serum creatinine to ESRD (Brenner *et al.*, 2001 and Lewis *et al.*, 2001).

There are many potential targeted investigational therapies available for treating DN. AGEs are being inhibited to decelerate the AGE deposition, and oxidative stress on the progression of DN. Pyridoxamine is being evaluated for scavenging ROS, inhibiting glycated proteins, and blocking the formation of protein products of lipoxidation (Turgut and Bolton, 2010). S-benzoyl thiamine monophosphate (a vitamin B1 derivative) is seen to reduce AGE formation and cytokine signaling leading to DN. Ruboxistaurin [a Protein Kinase C (PKC) inhibitor] is reported to decrease microalbuminuria (Tuttle et al., 2005). Pentoxifylline decreases proteinuria in diabetic patients (McCormick et al., 2008).

Transforming growth factor (TGF- β) regulates renal fibrosis, and anti-fibrotic agents are under active investigation (Decleves and Sharma, 2010). SMP-534 (inhibitor of p38) when studied in diabetic mice showed decreased progression of fibrosis (Sugaru et al., 2006). Many reports are available that use curcumin or its analog in treating DN complications due to epigenetic changes. In a report, curcumin was given to diabetic rats and decreased BUN and creatinine. Curcumin also decreased the levels of heat shock protein (HSP-27) and Mitogen-activated protein kinase (MAPK) (Tikoo et al., 2008). Curcumin analog C66 prevented DN in diabetic mice by downregulating JNK activation (Wang et al., 2015).

Globally, metformin is served in treating diabetes (Chen et al., 2010). But these synthetic drugs are loaded with side effects. The side effects of metformin are nausea, flatulence, diarrhea, ingestion, abdominal discomfort, and vomiting (Squibb, 2002 and Schwartz et al., 2006). Antihyperglycemic drugs like GLP-1 receptor agonists and alpha-glucosidase inhibitors also have similar side effects. DPP-4 are oral antidiabetic drugs that work by stopping the degradation of incretin hormone secretion, glucagon-like peptide-1 (GLP-1). When DPP-4 was used along with other drugs in combinational therapy it caused hypoglycemia (Gooben and Graber 2012). Sulphonylureas (SU) are another class of drugs given to diabetic patients. It works by binding to the receptor of sulphonylurea (SUR-1) potassium channels which is a subunit of beta cells of the pancreas and causes its

closure. As a result, the depolarized membrane opens voltage-dependent calcium channels, with subsequent release of insulin (Aittoniemi et al., 2009). Side effects of SU are weight gain and hypoglycemia both of which are attributed to the unregulated release of insulin (Monami et al., 2014).

Considering these side effects, researchers are dependent on phytochemicals for the discovery of effective drugs with the least or no side effects hence phytochemicals are emerging as alternatives, and many of them have already been used to treat nephropathy (Figure 1.2). Nutriepigenetics is an exciting new field to understand the influence of dietary components in regulating epigenetic mechanisms. Phytochemicals are relatively safe, less toxic, and interact with multiple targets, preventing and curing deleterious complications including insulin resistance and hypertension. Pharmaceutical companies also rely on pure bioactive compounds for new drug development.

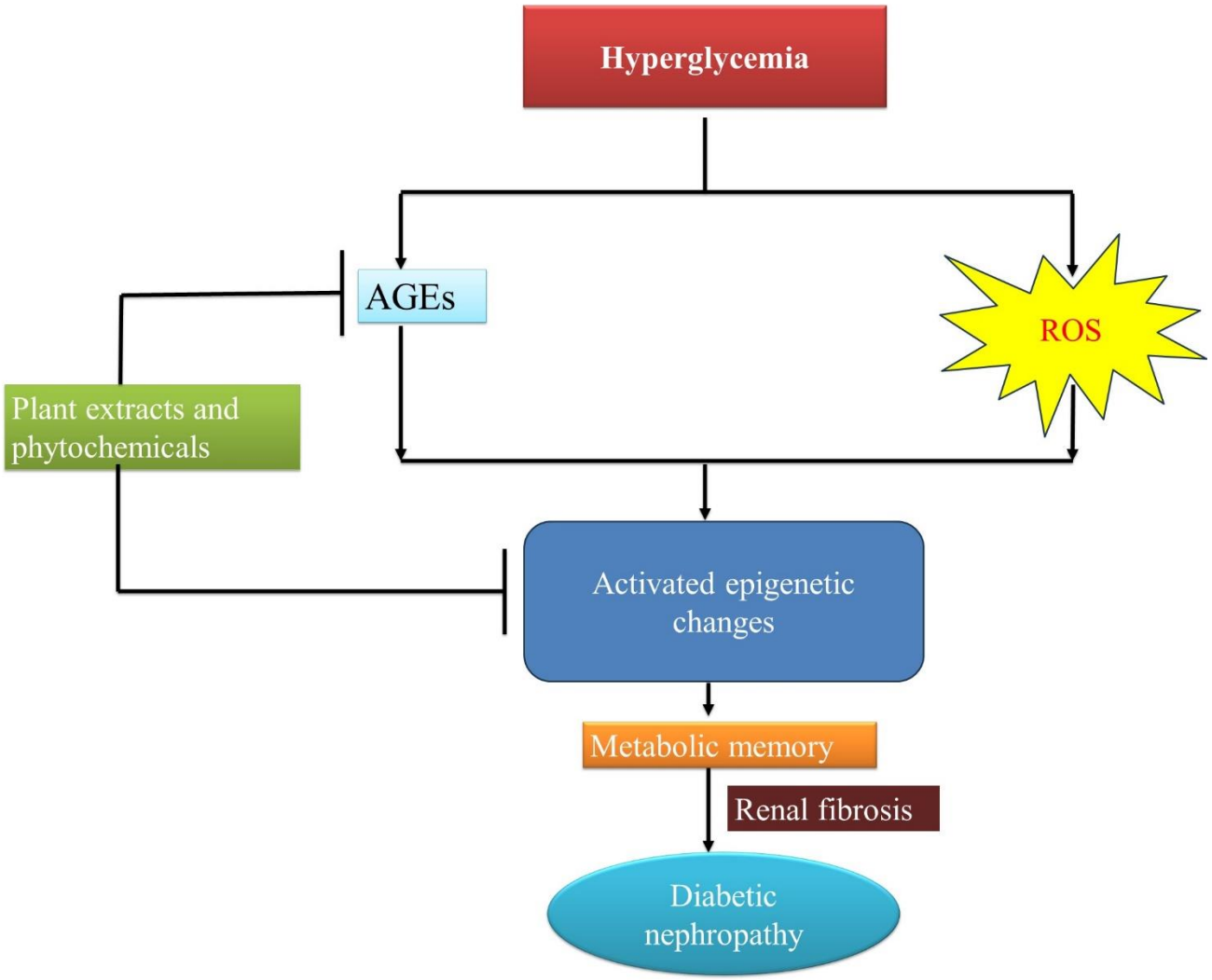


Figure 1.2: Schematic diagram shows the role of hyperglycemia-induced oxidative stress in regulating epigenetic changes and DN, further highlighting the protective effects of phytochemicals

Chapter 2

Review of Literature

The statistics of diabetes

International Diabetes Federation (IDF), states that around 536.6 million people globally are affected with diabetes, with a 10.5% prevalence rate. 75% of them live in low as well as middle-income nations. By 2045, it is anticipated that 783.2 million people will be diabetic (IDF, 2022). Most diabetic adults are from low or middle-income countries, hence causing 4 million deaths every year, and are common among people who are in the age group of 40-59 years. Diabetes also impacts the socioeconomic status of an individual who spends 2.3 times more than a healthy person (Kowluru et al.,2015). The total expenditure to manage and treat diabetes worldwide was around 727 billion USD dollars in 2017 putting the burden on economies mostly developing and underdeveloped countries and around 352 million people may develop T2DM.

T2DM affects vital organs of the body and complications associated with it are categorized as microvascular (like retinopathy, nephropathy, neuropathy, etc.) and macrovascular (coronary/peripheral artery disease, stroke, etc.) complications (Zhu et al., 2022). Nearly 40% of diabetic patients tend to acquire DN which leads to the dysfunction of the renal system and eventually changes to ESRD. Factors affecting DN mainly include ROS, persistent high glucose levels, increase in proinflammatory cytokines, AGEs, and alterations at the molecular level like altered PKB, AMPK, and thioredoxin interacting protein (TXNIP) (Yosri et al., 2022). Diabetes also affects transcription in specific tissues and numerous signaling pathways. It also leads to unusual expressions of several pro-fibrotic and pro-inflammatory genes that are known to cause DN (Kanwar et al., 2011). The condition gets worse as the DN progresses which makes the kidney impotent to clear toxins from the body (Li et al., 2004).

DN is a progressive kidney disease with an average occurrence of 3% per year during the first 10 to 20 years after diabetes onset (Magee et al 2017). T2DM is major contributor of ESRD and approximately 40-50% of all cases require renal replacement therapy and is a major contributing factor to the death of diabetic patients (Lopez et al., 2012; GABAlla and Farag 2013). DN develops in about 15-40% of Type I diabetic patients, whereas the incidence is highly variable ranging from 5-20% in the case of T2DM (Navarro et al., 2009; Lopez et al., 2012 and Lei et al., 2014).

Cellular mechanism of DN

Many factors causing DN are known to mankind few of them include persistent high levels of glucose, ROS, insulin sensitivity, growth factors, AMPK, mammalian target of rapamycin (mTOR), PKB, and TXNIP. In DN, there is an interplay between hemodynamic and metabolic pathways. Diabetes causes serious metabolic and hemodynamic alterations damaging the morphology and working of the kidney. Persistent hyperglycemia causes tissue injury and declining renal function through the generation of

AGEs. AGEs are formed from glucose extracellularly and dicarbonyls intracellularly. AGEs induce ROS production that is known to modulate various kinase activities and factors contributing to ECM pathology (Li and Gobe, 2006). Some of them are detailed below with how their stimulation is interlinked and they work by the activation of various pathways of DN (Figure 2.1).

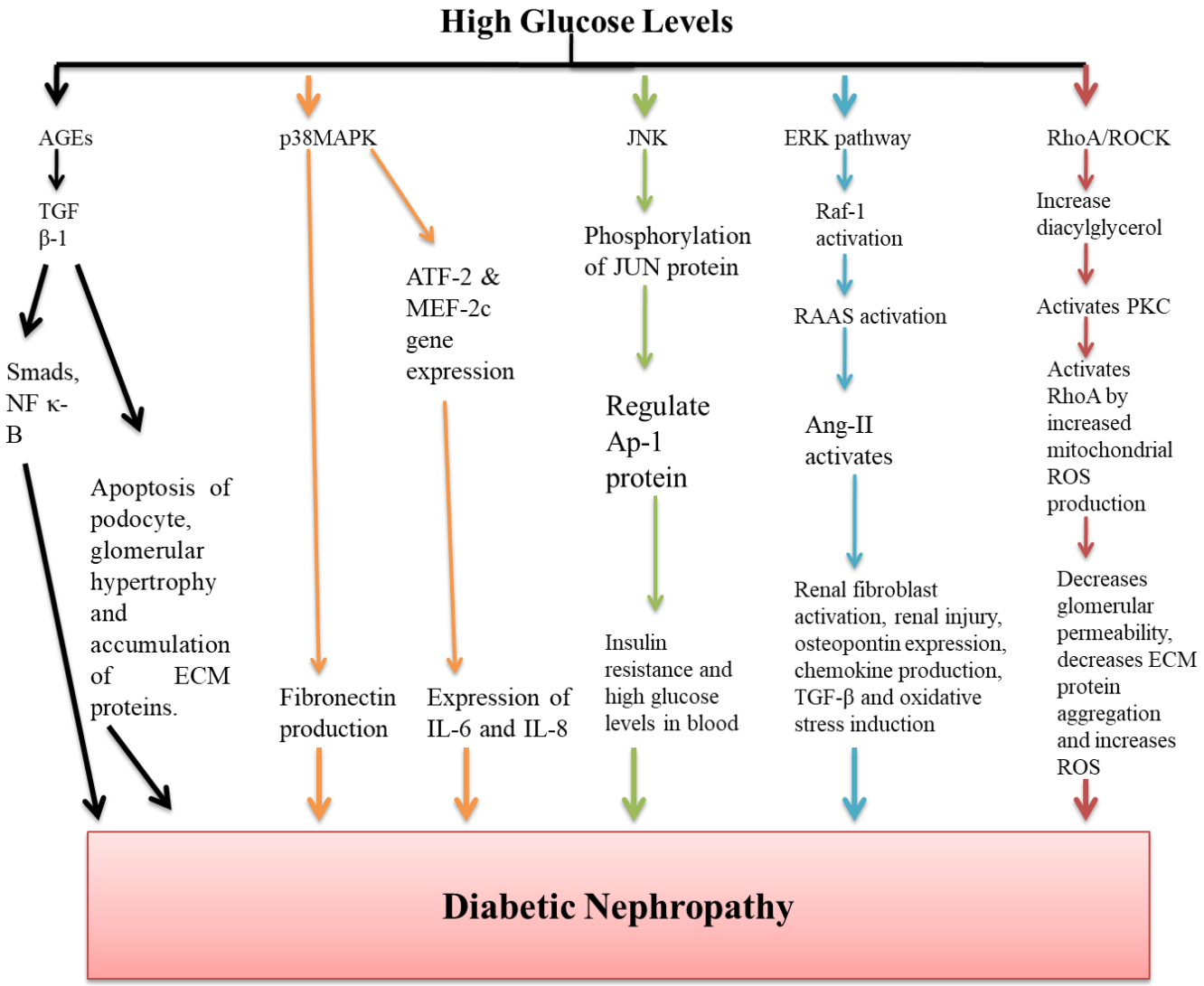


Figure 2.1: Different mechanisms responsible for causing DN. AGEs, p38MAPK, JNK, ERK, and RhoA pathways are activated under hyperglycemic settings which causes apoptosis of podocytes, ECM accumulation, increased ATF-2 gene expression causing fibronectin production, RAAS gets activated which cause renal injury and oxidative stress ultimately leading to DN.

Since the kidney is susceptible to these metabolic changes, under the diabetic condition it may cause severe organ damage. Diabetes may damage the kidney through various pathways like AGEs, abnormal PKC, genetic activation and expression, hexosamine pathway, myoinositol pathway, generating ROS, and the polyol pathway (Mesallamy et al., 2012). Reports also have shown that some glomerular abnormalities also include podocyte structure alteration and reduction in nephrin (Guo et al., 2023). Reports have also supported the fact that

monocytes, cytokines, chemokines, macrophages, growth factors are responsible for DN pathogenesis and its complications (Navarro and Mora., 2008 and Zhou et al., 2022).

PKC has been found to play a crucial role in the pathogenesis of DN (Rajab and Mohammad, 2022). Under the hyperglycemic condition, PKC is activated by diacylglycerol (DAG), an increase in phospholipase C, and intracellular Dag and Ca^{2+} (Inoguchi et al., 2003) leading to the dysfunction in endothelium increase in the expression of vascular endothelial growth factor (VEGF) and endothelin-1. Consequently, it changes the blood flow and permeability of capillaries (Inoguchi et al., 2003). Meanwhile, an increase in the expression of plasminogen activator inhibitor (PAI-1) and nuclear factor (NF-kB) leads to microangiopathy and tissue inflammatory response. Several reports suggest a decrease in renal abnormalities by PKC inhibitors emphasizing the role of PKC in the DN (Inoguchi et al., 2003).

Hyperglycemia impacts adversely every type of kidney cell like podocytes, endothelial cells, mesangial cells, tubular cells along with macrophages. It results in the production of AGEs and increases the levels of several growth factors like angiotensin II and TGF-β1 in renal cells. TGF-β signaling is activated by PKC, DAG, ROS, and AGEs (Leask and Abraham, 2004). TGF-β has the credit of being a major cytokine involved in the ECM pathophysiology in DN (Tang et al., 2022). Firstly, the binding of TGF-β to receptor type II trans-phosphorylates the type 1 serine kinase receptor, and later, it interacts with small mothers against decapentaplegic homolog 3 (Smad2 and 3), forming the Co-Smad4 complex (Schiffer et al., 2000) which is then translocated to the nucleus binding to the target genes of TGF-β like Jun B, collagen α1 (I), PAI 1, c-Jun and regulates their transcription. TGF causes the worsening of DN because it has profibrotic actions (Patel et al., 2018). There is an increase in the production of cell cycle genes, profibrotic genes, and proinflammatory cytokines. The interplay between signal transductions, PKC, PKB, and tyrosine kinases (non-receptor proteins) causes the activation of these proteins (Sanchez and Sharma, 2009 and Kanwar et al., 2011). Specific genes that regulate DN phenotypes vary according to the target cell type. The levels of TGF-β1 (profibrotic) are elevated in renal cells that are known to mediate apoptosis of podocyte, glomerular hypertrophy, and aggregation of ECM proteins that eventually cause DN (Kanwar et al., 2011 and Kato and Natarajan 2014). Macrophages are often responsible for inflammation in endothelial cells (Yang et al., 2020).

The MAPK pathway plays a central role in the pathogenesis of diabetic nephropathy. The MAPK pathway consists of three types of kinases: MAP3K (Mitogen-Activated Protein Kinase Kinase Kinase), MAPKK (Mitogen-Activated Protein Kinase Kinase), and MAPK. (Chang and Karin 2001). Responses happening in the renal cells are the result of the MAPK signaling pathway. These MAPKs are further classified into four subfamilies; namely (a) c-Jun N-terminal kinase (JNK1/2/3/) or Stress activated protein kinases (SAPK) (b) Extracellular-regulated kinase 5/Big mitogen-activated protein kinase (ERK5/BMK1), (c) extracellular signal-related kinase (ERK1/2) and (d) p38 mitogen-activated kinase (p38MAPK) (Maestroni et al 2005). When these factors are up or down-regulated they start different networks of versatile signal transduction, which in turn manages and governs a large nUMBer of cellular activities (Han et al., 2022). MAPKs regulate the transcription

of ECM genes mainly via activated protein-1 (AP-1) which is a heterodimer of c-FOS and c-Jun. Connective tissue growth factor (CTGF) is activated by TGF-β by transcription enhancer factor (TEF) and Smad (Peng et al., 2021). The signaling cascade discussed above has been proven with the help of *in vitro* cell culture systems in mesangial cells. Moreover, several *in vivo* studies carried out in mice reported elevated TGF-β activity. It was also observed that anti-TGF-β antibodies prevented mesangial matrix expansion, renal hypertrophy, increased fibronectin mRNA, and collagen expression in streptozotocin (STZ) induced mice (Ziyadeh, 2004). Reports have also shown that ACE inhibitors can improve renal damage by lowering TGF-β production, hence hypothesizing the relationship between hypertension and ECM pathophysiology in DM or DN (Wolf, 2006).

JNK is categorized into JNK1, JNK2, and JNK3 pathway that activates MAP3K by the involvement of cytokines as well as environmental stress (Zhao et al.,2012). Mitogen-activated protein kinase kinase kinase (MEKK1) is the most efficient JNK-activating MAP3K. Experiments (*in vitro*) have shown that tyrosine of JNKs gets phosphorylated by MKK4/7. Scaffold proteins regulate and activate JNKs to collect kinases that have been activated (Matteucci and Giampietro, 2000 and Raman et al., 2007). So, inhibition of JNKs can delay the onset of DN.

p38MAPK is also known to promote the gene expression of cyclic AMP-dependent transcription factor (ATF-2) and myosin enhancer factor (MEF-2C) which leads to the increased expression of interleukins (ILs), IL-6 promoting DN, and rate of glomerulonephritis (Navarro and Fernandez, 2008 and Chen et al., 2013). So based on experiments and reports published it is apt to say that deactivating p38MAPK can delay DN and vice versa.

Gorin et al., 2004 proved that hyperglycemia and growth factors are responsible to activate ERK pathways in various kidney cells. The activation of rapidly accelerated fibrosarcoma (Raf-1) by hyperglycemia subsequently activates a series of factors like RAAS and activates Ang-II, resulting in sufficient damage to kidney cells (Long et al., 2004). The hindrance of Ras homolog gene family, member A (RhoA)/Rho-associated protein kinase (ROCK) signaling pathways is also known to inhibit the DN. Hyperglycemia increases diacylglycerol hence activating PKC (Mehta et al., 2001). RhoA is activated by increased mitochondrial ROS generation (Komers et al., 2011). Moreover, RhoA is also known to decrease glomerular permeability, decrease ECM protein aggregation, and increase ROS which subsequently ameliorates DN progression (Nagai et al., 2022).

Growing evidence suggests that ROS induced by hyperglycemia is one of the common causes responsible for the development of complications in DM (Brownlee, 2001 and Ha and Lee, 2005). In general, it is produced and degraded in the body but when formed in higher amounts can cause severe tissue injury in organs. ROS family includes H_2O_2, hydroxyl radical, peroxynitrite, and superoxide anion generating free radicals causing renal injuries (Djordjevic, 2004). Various endogenous enzymes such as mitochondrial manganese SOD (MnSOD), cytoplasmic Cu/ Zn SOD(Cu/ZnSOD), and heme oxygenase-1(HO-1) (Koya et al., 2003) maintain ROS levels. ROS is generated *via* oxidative phosphorylation and small amounts by the nicotinamide adenine dinucleotide phosphate hydrogen (NADH/NADPH)-oxidase system (Kang and Hamasaki, 2003 and Li and

Shah, 2003). Apart from this several other pathways/molecules generate ROS like AGEs and NFκB. An increase in the production of ROS modulates the activity of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), MAPK, and PKC along with various transcription factors increasing the expression of ECM and eventually causing fibrosis and ESRD (Craven et al., 2001 and Ha and Lee, 2005).

Accumulating evidence from diabetic animal model studies has shown the accretion of macrophages in the kidney resulting in the deterioration of renal function (Lopez et al., 2012). Two subtypes of macrophages are involved in DN progression, M1 macrophages elevate inflammatory response by the expression of TNF(TNF), IL, and interferon (IFN γ) and also upregulate the production of ROS whereas M2 macrophages promote remodeling by anti-inflammatory cytokines expression and superoxide anions production (Lopez et al., 2012).

Convincing data now relate DN as an inflammatory disease describing the role of immune cells in DN. The hyperglycemia-induced lesion can be a cause of lymphocyte infiltration and accumulation in the kidneys of DN (Lopez et al., 2012). In animal models, an increase in the cluster of differentiation (CD) 4 and CD8 cells was observed in glomeruli (Moon et al., 2012) establishing the role of T cells in DN. Numerous reports have shed light on the fact that circulating CD8 is correlated with albuminuria (Montigny et al., 2020). Furthermore, patients who had urinary albumin excretion displayed a higher amount of a chemokine MCP-1 (El Mesallamy et al., 2012).

Inflammatory cytokines have now been recognized as the molecules that lead to ESRD (Figure 2.2). Reports have elucidated (Navarro and Mora, 2008) increased renal expression of IL-1 in in vivo models of DN (Navarro et al., 2006 and Gu et al., 2019), and it is related to the increased expression of several adhesion molecules that enhance the production of vascular cellular adhesion molecule-1 (VCAM-1) and Intercellular adhesion molecule-1 (ICAM-1). In diabetic models, IL-1 has been shown to cause prostaglandin E2 and hyaluronic synthesis that leads to changes in the glomerular hemodynamics further leading to DN progression (Jones et al., 2001). Pathological changes cause IL-1 to increase the expression of chemokines that in turn initiate the synthesis of ECM in the mesangium (Lopez et al., 2012). IL-6 concentration is also increased in DN and it directly affects the membrane thickening in renal glomeruli (Navarro and Mora, 2008 and Lopez et al., 2012).

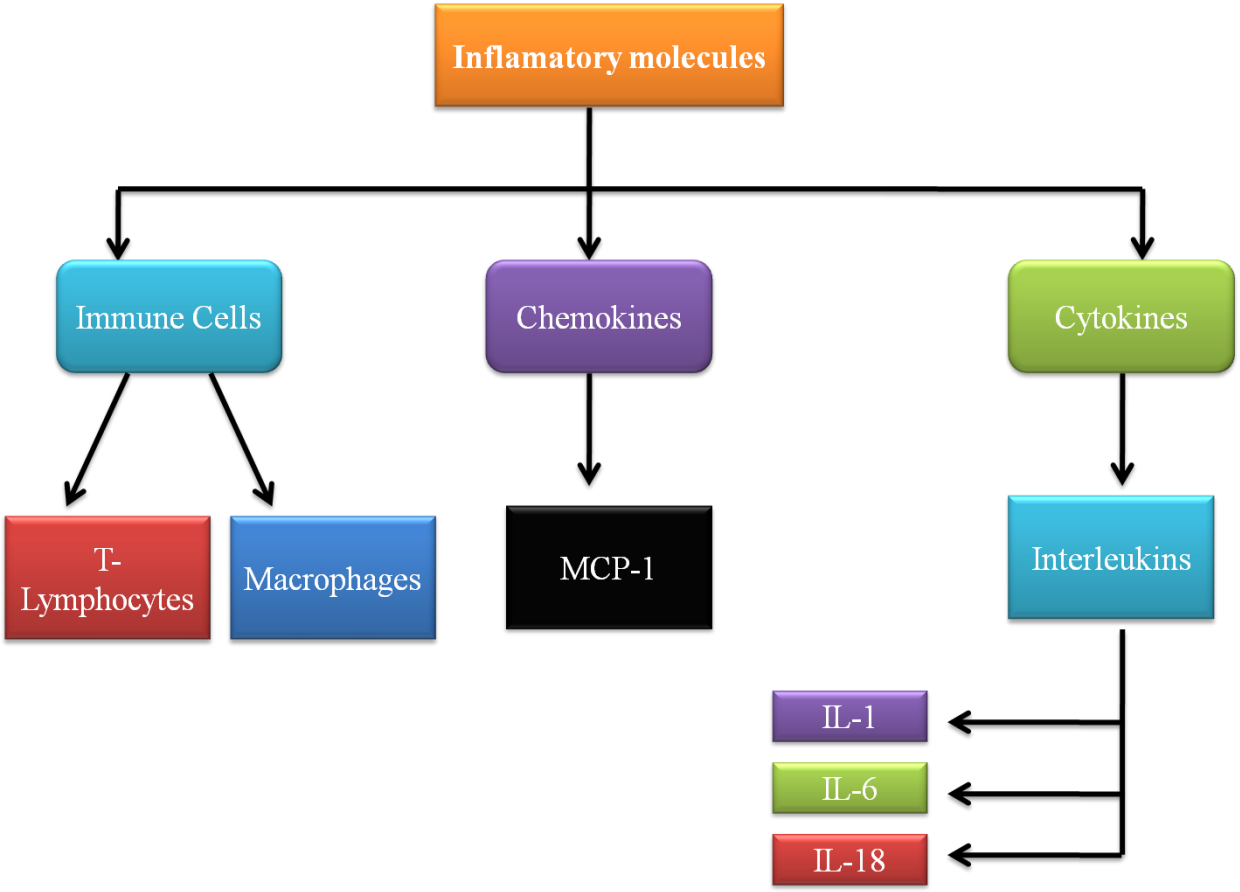


Figure 2.2: Inflammatory cells in the advancement of DN.

Several clinical research data reported increased concentration of TNF-α in the urine of DN patients and iTNF-α produced by intrinsic kidney cells are capable of producing a variety of effects on the different renal structure. It also increases endothelium permeability and the new formation of ECM, ROS production, disturbances in blood flow, and increases sodium reabsorption are several attributes associated with TNF-α (Navarro et al., 2009). Additionally, the mRNA coding for TNF-α is also found to be elevated in proximal tubule cells in rats (Dipetrillo et al., 2003; DiPetrillo and Gesek 2004 and Navarro et al., 2005). This support the fact that TNF-α is involved in renal hypertrophy and hyperfunction, the two major alterations in the progression of DN (Dipetrillo et al., 2003 and DiPetrillo and Gesek 2004;) (Figure 2.3).

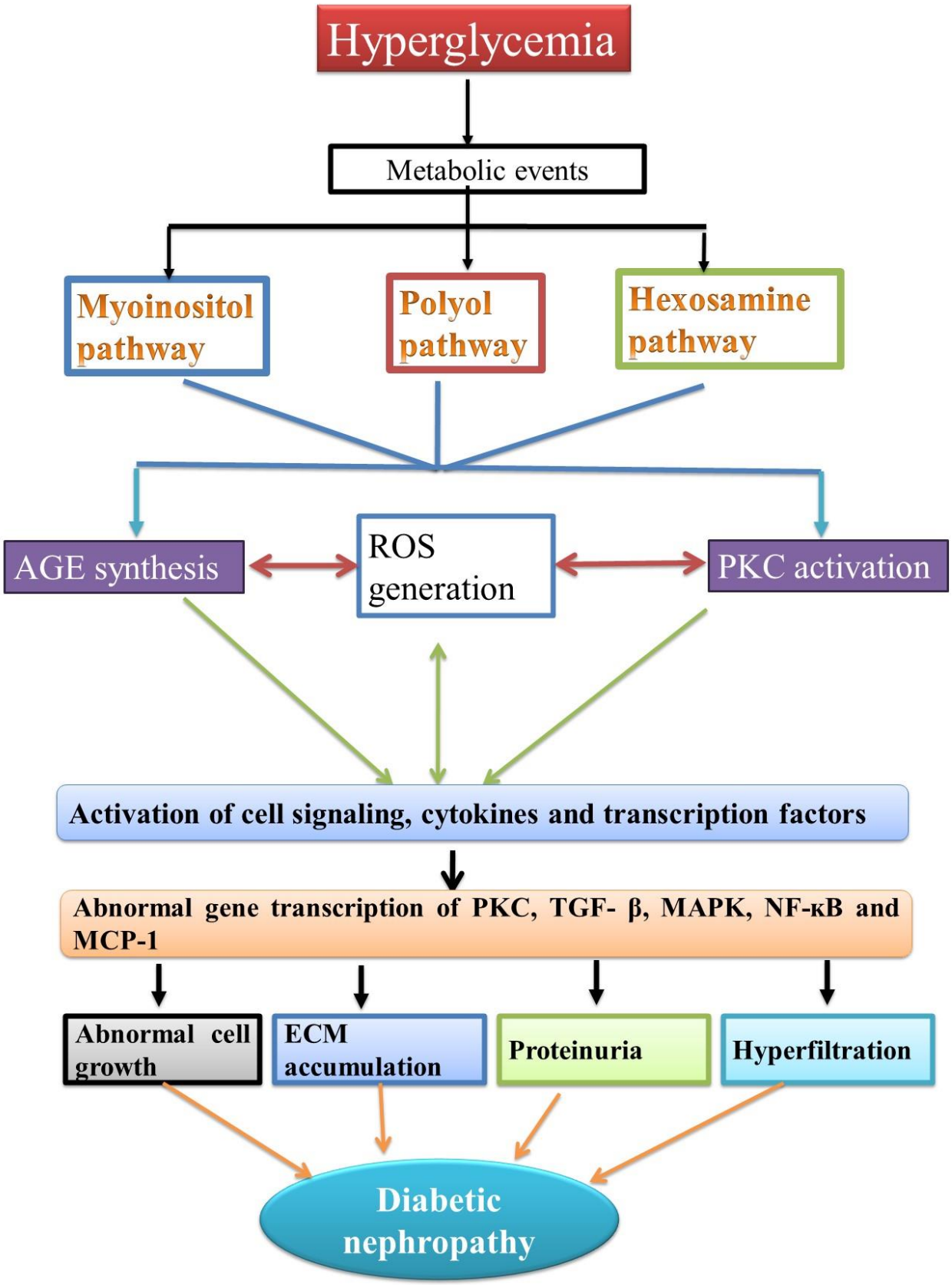


Figure 2.3: Different pathways involved in DN progression. ROS generation cause the synthesis of AGEs and activates PKC which in turn causes cell signaling activation leading to abnormal cell growth, ECM accumulation, proteinuria ultimately causing DN

Pathophysiology of "GM Phenomenon"

According to Costantino et al., 2015, the exact underlying molecular mechanisms of "GM" remain unknown. There are several reasons postulated for the causality connection between hyperglycemia and memory phenomena (Bianchi et al., 2013). These reasons implicated that high levels of glucose can have adverse effects on cardiovascular systems in different ways; like the production of AGEs and free ROS, which eventually causes proteins, nucleic acids, and lipids to glycate (Cao et al., 2019; Yang et al., 2019; Egaña-Gorroño et al., 2020 and Dozio et al., 2021). The presence of AGEs can deteriorate body cells and their functioning as they arouse pathological angiogenesis and vascular hyperpermeability, and inhibit DNA synthesis by activating VEGF and PAI-1 by the interaction with AGEs receptor (RAGE) (Chen et al., 2020; Nakano et al., 2020; Takino et al., 2021; Tsuruhisa et al., 2021 and Weng et al., 2021).

Hence, the products of glycation pathways are important mediators in GM and complications related to diabetes. According to Genuth et al., 2015, AGEs are correlated with retinopathy independent of Hb1Ac levels, and the risk of nephropathy associated with AGEs is less than neuropathy and retinopathy. Hence, the generation of AGE by glucose-activated epigenetic changes increases the expression of MCP-1, NF-κB and VCAM-1 in cells that cause inflammation, tissue injury and many more. So, it can be concluded that accumulation of AGE may cause an aberrant gene expression at times when high glucose levels have been controlled (Bhagat and Zarbin 2019).

Many animal models have been developed to mimic GM. In these studies, end reactive product accumulation, the mechanism of oxidative stress and epigenetic mechanisms for modifying gene expression have been fully elucidated (Sarras et al., 2015). There are reports to support the fact that high glucose levels can cause an anomaly in the action of PTM, DNA methyltransferase (DNAMT), and miRNA levels which are altered in different cells like; cardiomyocytes, retina, and renal cells. Aggregation of AGEs and OFRs are the factors leading to GM in endothelial cells (Costantino et al.,2015). Therefore, epigenetic modifications are apparently becoming important modulators of inflammatory genes and antioxidant genes thereby leading to inflammation and cell alteration.

Multiple results have suggested that microvascular and macrovascular diabetic complications may also be due to alterations in multiple epigenetic modifications like histone PTMs, DNAme, and miRNA (Ling et al., 2009; Reddy and Natarajan, 2011; Jin et al., 2019; Lu et al., 2021; Pang et al., 2021; Ismail et al., 2022; Ko et al., 2022, and Zhou et al., 2022).

Epigenetics in GM

Epigenetic processes have been linked to GM in diabetic complications such as DKD. From the DCCT/EDIC trials, data showed that the T1D patients receiving regular treatment had progressing nephropathy and high H3K9Ac levels in the inflammatory genes of the monocytes when compared to the patients receiving strict

glycemic control. This study, hence, discovered a link between HbA1c and H3K9ac, implying that H3K9ac may have a role in GM in T1DM patients due to hyperglycemia-induced prolonged chromatin relaxing at vulnerable genomic areas via histone hyperacetylation. Experimental evidence also suggests that epigenetic pathways play a role in GM. According to one study, diabetic db/db mice's VSMCs expressed more inflammatory genes than non-diabetic heterozygous db/ + mice's VSMCs. Even after the VSMCs had been cultivated for multiple passages, the anomaly in histone methylation remained. MiR-125b, which targets SUV39H1, was also upregulated in VSMCs proving miRNAs an important part of GM (Villeneuve et al., 2008,2010).

Epigenetics and role in DN

Epigenetic changes are reversible and inheritable (Lind et al., 2018). Being able to get inherited, these epigenetic marks possess the ability to expose future generations to these disorders (Wang et al., 2012). Epigenetic modifications are classified into 3 categories: (a) DNAme (b) Histone modifications and (c) miRNA.

(a) DNA methylation: It is basically carried out with the help of enzymes called DNMTs (Villota et al., 2016). DNAme has been extensively studied for metabolic diseases and concluded that diet and environment is a contributing factor to epigenetic modifications at transposable elements, imprinted genes, and repeated elements in genomic DNA to regulate disease progression (Jirtle and Skinner 2007). DNAme regulates various pathophysiological processes. But how much it is involved in DN progression is not well studied (Williams and Schalinske, 2012). Many studies showed that aberrant DNAme plays a role in downregulating energy metabolism, and insulin expression in skeletal muscles underlying diabetes pathogenesis (Yang et al., 2011 and Yang et al., 2012). During an assessment of the DNAme pattern in the blood of a diabetic patient with DN, a differential methylation pattern on genes was found to be initially linked with DN (Bell et al., 2010). On the contrary, studies that were done on renal cell culture from diabetic models or DN models didn't show specific changes in DNAme pattern (Reddy and Natarajan 2011). Researchers found that when Ras protein activators like 1 (RASAL1) were hypermethylated, it resulted in retrovirus-associated DNA sequences (RAS) activation in fibroblasts that lead to proliferation and fibrosis (Bechtel et al., 2010).

DNMT1, DNMT3A, and DNMT3B are the three enzymes that catalyze DNAme at CpG dinucleotides in mammals (Liang et al., 2002 and Strausmann et al., 2009). To maintain DNAme in mammals during replication DNMT1 is used while *de novo* methylation patterns are provided by DNMT3A and DNMT3B (Okano et al., 1999 and Ooi and Bester, 2008). It has been reported that a gene is activated under low methylated DNA while on the other hand transcription is repressed in hypermethylated DNA status (Fan and Zhang, 2009). DNAme is reversible (Wu and Zhang, 2010 and Chen and Riggs, 2011). Reports earlier have shown that cells under hyperglycemic conditions induced DNAme changes accompanied by pathways activation associated with DN progression (Cooper and El-Osta, 2010 and Pirola et al., 2011). In the backdrop of diabetes, it is studied as a

transgenerational inheritance where environment and diet may affect epigenetic modifications to regulate the pathogenesis of the disease (Jirtle and Skinner, 2007, and Dolinoy and Jirtle,2008).

A comparative study done between diabetic patients with and without DN results showed that there was differential methylation at several genes, UNC13B being one of them which was previously linked to DN progression (Sapienza et al., 2011). DNAme causes TGF-β and fibrosis activation. When RASAL1 was hypermethylated it led to increase RAS activation ultimately causing fibrosis. When the tubules of DN patients were micro-dissected for DNAme patterns, it showed differently methylated genes involved in fibrogenesis (Hasegawa et al., 2013). By the sequencing, it was also revealed that when EC was exposed to high glucose ambiance it led to changes in DNAme of key genes that caused endothelial cell dysfunction (Pirola et al., 2011). In diabetic patients with and without DN, it was observed that apart from different methylating patterns there were changes in the genes that control mitochondrial function for kidney disease (Swan et al., 2015).

(b) Histone Modifications

These are basic proteins that help in the packaging of DNA tightly inside structural units called a nucleosome. Nucleosome has a pair each of 4 histone proteins (H2B, H3, H2A, and H4) (Ramakrishnan 1997 and Mariño-Ramírez et al., 2005) which form the core of nucleosomes and can undergo several PTMs often known as "histone marks". These modifications may be phosphorylation, methylation, ubiquitination, ADP-ribosylation, acetylation, etc (Chen et al., 2017). Kouzarides, 2007 and Zhou et al., 2011 reported that the PTMs of histone proteins also regulate gene expression. Histone modifications like serine phosphorylation, lysine methylation (Kme), and lysine acetylation (Kac) are more extensively studied and are mostly present at amino-terminal tails. Histone lysine acetylation, when employed at the site of gene promoters, is known to upregulate transcriptional activation and vice versa. But HKMe can either upregulate or downregulate gene expression, depending upon the amino acid residue attached to it or the nUMBer of methyl residues added, for instance: tri (Kme3) methylation, mono (Kme1) and di (Kme2). Kouzarides, 2007 reported that histone modifications related to transcriptionally active regions are H3K36me2/3 and H3K4me1/2/3, on the other hand, histone modifications related to repressed domains are H3K27me3, H4K20me3, and H3K9me3. Gene promoters that are transcriptionally active are filled with H3K4me2, H3K9ac, and H3K4me3 whereas the regions that are transcribed are filled with H3K79me3 and H3K36me3. The inactive gene promoters, on the other hand, are enriched with H3K27me3 and H3K9me3 (Zhou et al., 2011). HAT like p300 and CBP mediates HAc. On the contrary histone deacetylase (HDAC) like sirtuins and HDAC1-11 eliminates acetylation by acting as a co-repressor. Lysine methylation is regulated by HMTs (histone lysine methyltransferases) and eliminated by KDMs (lysine demethylases) (Klose et al., 2007 and Zhou et al., 2011). Reddy and Natarajan (2011) have reported that the expression of many diabetes and obesity-associated genes is affected in organs like the liver, pancreas etc. if there is any change in histone PTMs.

TGF- β has an important role and contributes to DN by altering different histone modifications and expressing several genes like ECM, fibrotic, and cell cycle inhibitors (Ziyadeh and Sharma, 2003; Kanwar et al., 2011; Forbes and Cooper, 2013 and Kato and Natarajan, 2014). Activation of transcription factors Smads2/3/4 is regulated by TGF-β by its collaboration with chromatin remodelling factors and HATs (Kanwar et al., 2011). Some studies have demonstrated these mechanisms and altered histone Kme and Kac levels in the mesangial cells of rats with high glucose and TGF-β (Meng et al., 2016). They result in the elevated H3K9/14ac near protein-specific binding sites (SP1) and Smad by recruiting CBP and HATs p300 to the gene promoters encoding for plasminogen activator inhibitor type 1 (PAI-1) and p21 (Yuan et al., 2012). Active lysine methylation sites (H3K4me1/2/3) and repressive sites (H3K9me2/me3) at their promoters induced the fibrotic genes in mesangial cells of rats by TGF-β (Sun et al., 2010). When the mesangial cells of rats were exposed with high glucose it led to familiar changes in histone modifications and control to the recruitment of cell cycle and fibrotic promoters (Yuan et al., 2013). It was demonstrated also that TGF-β acts as a mediator to block epigenetic changes due to high glucose levels, suggesting an important role of epigenetics in high glucose-induced pathological gene expression in mesangial cells (Sun et al., 2010). A study demonstrated that a decrease in H3K27me3 levels and an increase in RNA polymerase II recruitment and H3K4me2 resulted in the expression of DN-related genes in rat and mouse models of DN (Komers et al., 2013).

(c) miRNA

miRNAs are small non-coding RNAs (ncRNAs) that are generally 20-22 nucleotides long and known for gene expression regulation through PTMs. These are ubiquitously expressed in animals and in plants (Friedman et al., 2009 and Hill and Tran, 2021). They first came into light in the year 1993 when the expression of protein lin-14 was seen to be regulated by the product of the *lin-4* gene in *Caenorhabditis Elegans* (Lee et al., 1993). Generally, miRNA work by deregulating the gene expression by aberrant base pairing to the 3'-untranslated region (3'UTR) of specific RNA by either degrading mRNA or by repressing translation (Bartel, 2009). More than 60% of the genes encoding for protein are regulated by miRNA (Ha and Kim, 2014 and Daugaard and Hansen 2017). It would be apt to say that miRNAs are involved in nearly all pathophysiological and physiological pathways. Improper functioning in miRNA has been reported to cause many diseases like diabetes-related complications, cardiovascular diseases, and cancers to name a few (Rupaimoole and Slack, 2017; Tana et al., 2017; Paul et al., 2018 and Ramassone et al., 2018). The function, biogenesis, mechanism, and role in DN of miRNA have been outlined in many studies (Finnegan and Pasquinelli, 2013; Libri et al., 2013, and Melo and Melo, 2014).

There are many miRNAs present in the renal area that control several functions of renal physiology (Tian et al., 2008 and Hou and Zhao, 2013). With the help of scientific advancements, several miRNAs have been recognized that are present in kidneys. The involvement of miRNAs in renal failure comes from the report that shows loss of Dicer activity due to podocyte injuries leads to glomerulosclerosis, proteinuria, and foot process effacement (Harvey et al., 2008). Many clinical trials are present that have shown the profound role of different

miRNAs in the progression of DN (Khella et al., 2013; Distefano et al., 2013; Wu et al., 2014; Kato and Natarajan, 2015; Eissa et al., 2016 a & b and Dewanjee and Bhattacharje, 2018). The first miRNA reported to be linked with DN is miR192 (Kato, 2006) and since then researchers were drawn towards miRNAs revealing their impacts on signaling pathways leading to nephropathy. Kato et al, 2006 have been credited with discovering a giant cluster of nearly 40 miRNAs that were present in non-coding RNAs (Kato et al., 2016). Assmann et al., 2018 did a systematic review recently and found that 6 out of 151 miRNAs namely: miR-29a-3p, miR-21-5p, miR-192-5p, miR-126-3p, miR-342-3p, and miR-214-3p were dysregulated in these studies which regulate innate immune response under type1 diabetic conditions.

Inflammation plays a crucial role in the advancement of DN (Donath, 2014 and Pollack, 2016). miRNAs' role in regulating inflammation is being studied to cope with the progression of DN. miRNA expression can be induced by TGF-β in glomeruli and mesangial cells in mice where these miRNAs mediate the expression of key fibrotic genes known for progressing DN (Mogyorosi and Ziyadegh, 1999). The expression of several miRNAs was found to be elevated in mouse mesangial cells when given TGF-β1 to the diabetic models of a mouse having DN (Kato et al., 2007 and Park et al., 2013). miR-27a used Peroxisome proliferator-activated receptors (PPAR-γ) and β-catenin signaling to cause podocyte injury (Zhou et al., 2018). When miR-27a was ablated caused reduced mesangial cell proliferation and antagomir of miR-27a ameliorated urine protein excretion (Wu et al., 2016). The role of miR-29c is studied by Zhao et al., 2016 in regulating the levels of tristetraprolin which is an anti-inflammatory protein. He found that the plasma samples taken from nephropathic elevated patients had increased levels of miR-29c. The upregulation of miR-29c was directly proportional to cytokines and inversely to tristetraprolin. This showed that miRNA had a direct role in exacerbating DN-associated inflammation (Guo, 2017). Natarajan's group also worked on similar lines to elucidate the effect of miR-146a, which is a very well-known anti-inflammatory miRNA that modulated the progression of DN. They performed experiments on miR-146a deficient mice that eventually developed glomerular hypertrophy, fibrosis, and proteinuria. An enormous increase in proinflammatory cytokines like IL-1β, MCP-1, and TNF-α, was observed suggesting the probable role of miR-146a against DN by hindering inflammatory pathways (Bhatt, 2016).

miR-21 is one of the rigorously studied miRNAs that showed several contradicting results. When miR-21 was over-expressed, it ameliorated albuminuria (Zhang et al., 2009 and Gomez et al., 2015). The above finding was also substantiated by Lai et al., 2015 in their report along with the downregulation of the miR-21 enhanced proteinuria and enhanced ECM aggregation. A study on the OVE26 type 1 diabetes mice model showed overexpression of miR-21 in the renal cortex. It activated Akt and other factors leading to DN (Dey et al., 2011 and Bera et al., 2017). TGF-β1 caused overexpression of miR-214 that activated Akt so that cell death can be reduced in THP1 cells (Denby et al., 2014). miR-214 when inhibited led to downregulated fibrotic gene expression and hence ameliorated fibrosis and glomerular hypertrophy (Wang et al., 2016). In db/db mice miR-21 inhibitor was used to inhibit miR-21 activity that increased the signaling of TGF-β1 (Zhong et al., 2013).

miR-192 can activate the Akt pathway hence signaling TGF-β1 downstream (Mu et al., 2013). miR-192 was overexpressed in renal vascular endothelial cells that were treated with high glucose and in glomeruli of db/db mice (Long et al., 2011). When miR-30 is down-regulated it leads to the progression of DN as CTGF is upregulated (Wu et al., 2014). When miR-93 was down-regulated it led to an increase in VEGF-A a proangiogenic target (Long et al., 2010). Let-7 was found to be downregulated in many cancers and accumulated report reveals that TGF-β downregulated the Let-7 in renal cells hence upregulating TGF-β1-R1 along with collagens (Wang et al., 2014). ROS are major contributors to DN. miRNAs like 146-a and 25 targets the Nox4 gene that is involved in DN progression (Sedeek et al., 2012).

There are few studies present that show that miRNAs are also involved in the progression of DN (Figure 2.4) through RAS, AGE, and oxidative stress pathways. By conditionally deleting Dicer in mice that are specifically expressed in renal cells, Sequeira-Lopez for the first-time reported miRNA in the maintenance of juxtaglomerular (JG) cells (Sequeira-Lopez et al., 2010) that resulted in a decrease in JG cells hence decrease in blood pressure and anomaly in renal functions. A study shows the ability of AngII to regulate miRNAs like miR-132/129-3p/132-5p/212/29b in HEK293N cells that were stimulated to overexpress AT1R (Jeppesen et al., 2011). High glucose levels form AGEs due to the Maillard reaction and AGEs, in turn, liberate ROS. Alternatively, AGEs can also trigger proinflammatory genes causing glomerular injuries (Cooper, 2001 and Gu et al., 2006). miR-214 was upregulated in monocytes of DN patients and in THP-1 cells that were treated with AGEs. Since PTEN is one of the target genes for miR-214 when pre-miR-214 was up-regulated it delayed the cell death of THP-1 cells (Li et al., 2011). miR-200 targets ZEB1/2 hence mediating the production of renal fibroblasts as it's a transcriptional repressor of E-cad (Korpal et al., 2008 and Park et al., 2008).

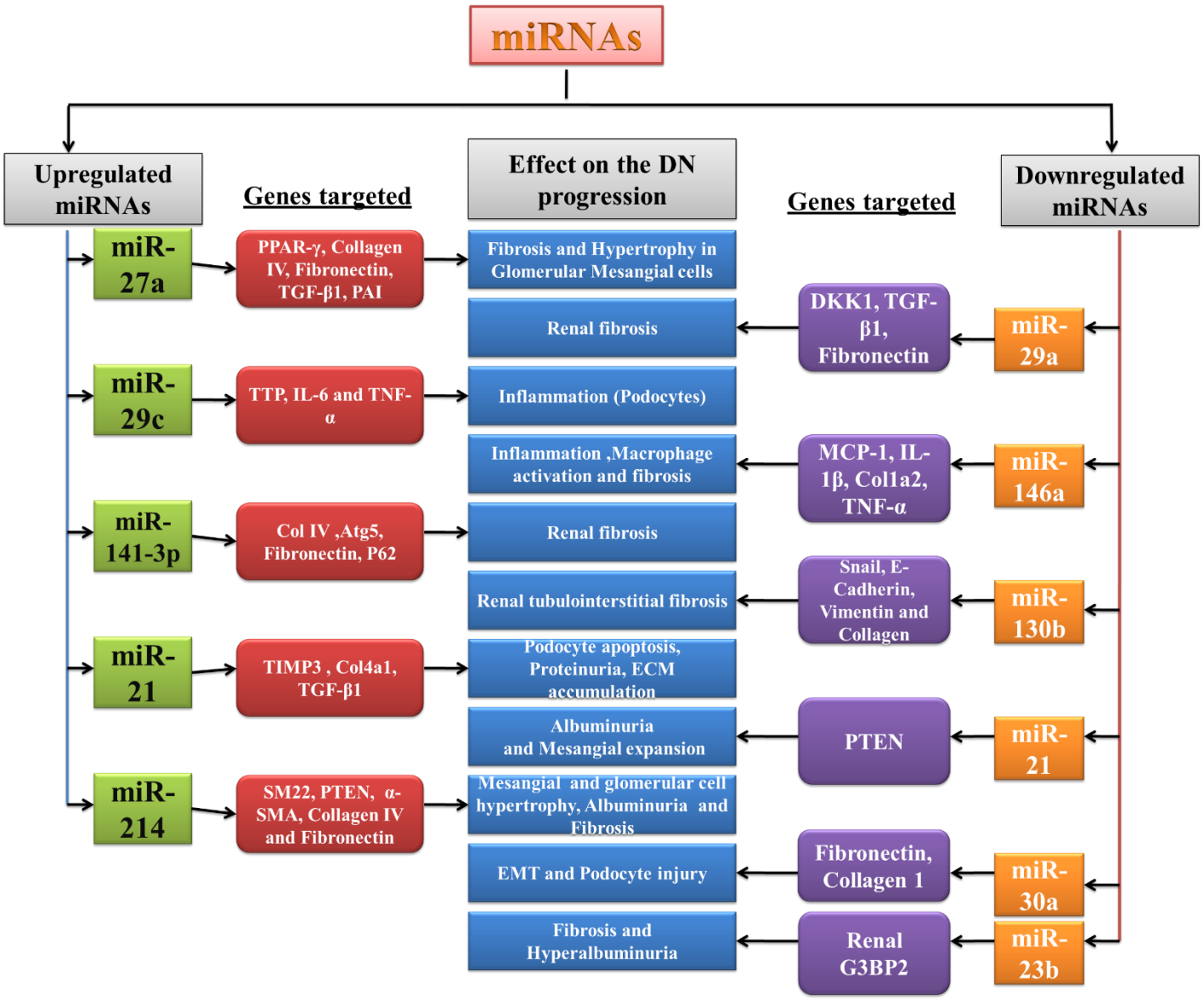


Figure 2.4: miRNAs involved in DN progression. The picture depicts the role of upregulated and downregulated miRNAs targeting different gene expressions and effects on DN progression.

Diagnosis of DN

DN is characterized by microalbuminuria and hyperfiltration followed by worsening in derangements of tubule interstitial and glomerular compartments. Clinically, DN is classified by the amount of proteinuria i.e. > 0.5 g/24 h, and this stage is classically known as overt nephropathy, macroalbuminuria, proteinuria, and clinical nephropathy that usually precedes a fall in glomerular filtration rate (GFR). Patients who progress to macroalbuminuria are more likely to develop ESRD. Other pathological changes seen in DN are hyalinization of arterioles, microaneurysm, and thickening in the branches of intrarenal arteries causing an anomaly in the regulation of glomerular microcirculation that can multiply the chances of renal damage (Mason and Wahab, 2003). When studied under electron microscopy, the worsening of glomerular lesions was found related to Urinary Albumin Excretion (UAE) and GFR (White and Bilous, 2000 and Caramori et al., 2002).

DN has been characterized into different stages based on the UAE values of micro and macroalbuminuria. It is recommended to use early morning urine samples to avoid misleading effects. The cut-off values are derived as per the American Diabetes Association for diagnosing the main clinical feature of each stage as set up by (Parving et al., 1982). The UAE cut-off values for determining the presence of albumin in urine and clinical features are presented in Table 2.1.

Table 2.1: UAE cut-off values for determining the presence of albumin in urine and clinical features:

Cut-off values for UAE	Clinical reference	Stage
≥200 µgm/min	High blood pressure.	Macroalbuminuria
≥300mg/24h	Increase in cholesterol, LDL, and Triglyceride levels.	Macroalbuminuria
>300mg/g	A continuous decline in GFR.	Macroalbuminuria
20-199 µgm/min	Increase in the levels of blood pressure	Microalbuminuria
30-299 mg/24h	Increase in cholesterol, LDL, and Triglyceride levels.	Microalbuminuria
30-299 mg/g	Anomalies in endothelial functioning, retinopathy, CAD, stable GFR, increase in cardiovascular mortality	Microalbuminuria

Several reports have suggested that the risk of developing DN and cardiovascular disease starts even when UAE values are still within the normal range (Gerstein et al., 2001). Immunoassays are also routinely exploited to detect albuminuria. High-performance-liquid chromatography (HPLC) is also used to estimate albumin presence in (Comper et al., 2004). In measuring microalbuminuria in a situation where UAE measurements are not available, semi-quantitative dipstick measurement like Micral test II needs to be done (Molitch et al., 2004 and Koroshi, 2007). Qualitative proteinuria detection (Sacks et al., 2002) or quantitatively measuring protein in the spot urine sample is another approach to detect albuminuria (Zelmanovits et al., 1998 and Bert et al., 2003). Several biomarkers like neutrophil gelatinase-associated lipocalin (NGAL), Immunoglobulin G (IgG), Urinary transferrin, and TNF-α are also employed in detecting DN (Zhang et al., 2019).

Screening should start immediately as soon as a person is diagnosed with type 2 diabetes (ADA, 2004) as nearly 7% of patients already have microalbuminuria at that time (Adler et al., 2003). Whereas, screening for type1

diabetes patients should be done after 5 years of diagnosis. Although some patients have decreased GFR even with a normal UAE range (MacIssac et al., 2004). Accumulating shreds of evidence suggest that normal albuminuria does not play a role in protecting against the decrease in GFR in both type 1 and 2 diabetic patients. GFR can be estimated by many techniques like ^{51}Cr-EDTA, insulin clearance, iohexol, and ^{125}I-iothalamate (Seegmiller et al., 2018). Taken together, proper screening of DN, UAE, and GRF should be measured in a structured regular manner.

Treatment for DN

The basis to prevent DN is to control blood sugar, blood pressure, dietary protein, and lipid intake (Figure 2.5).

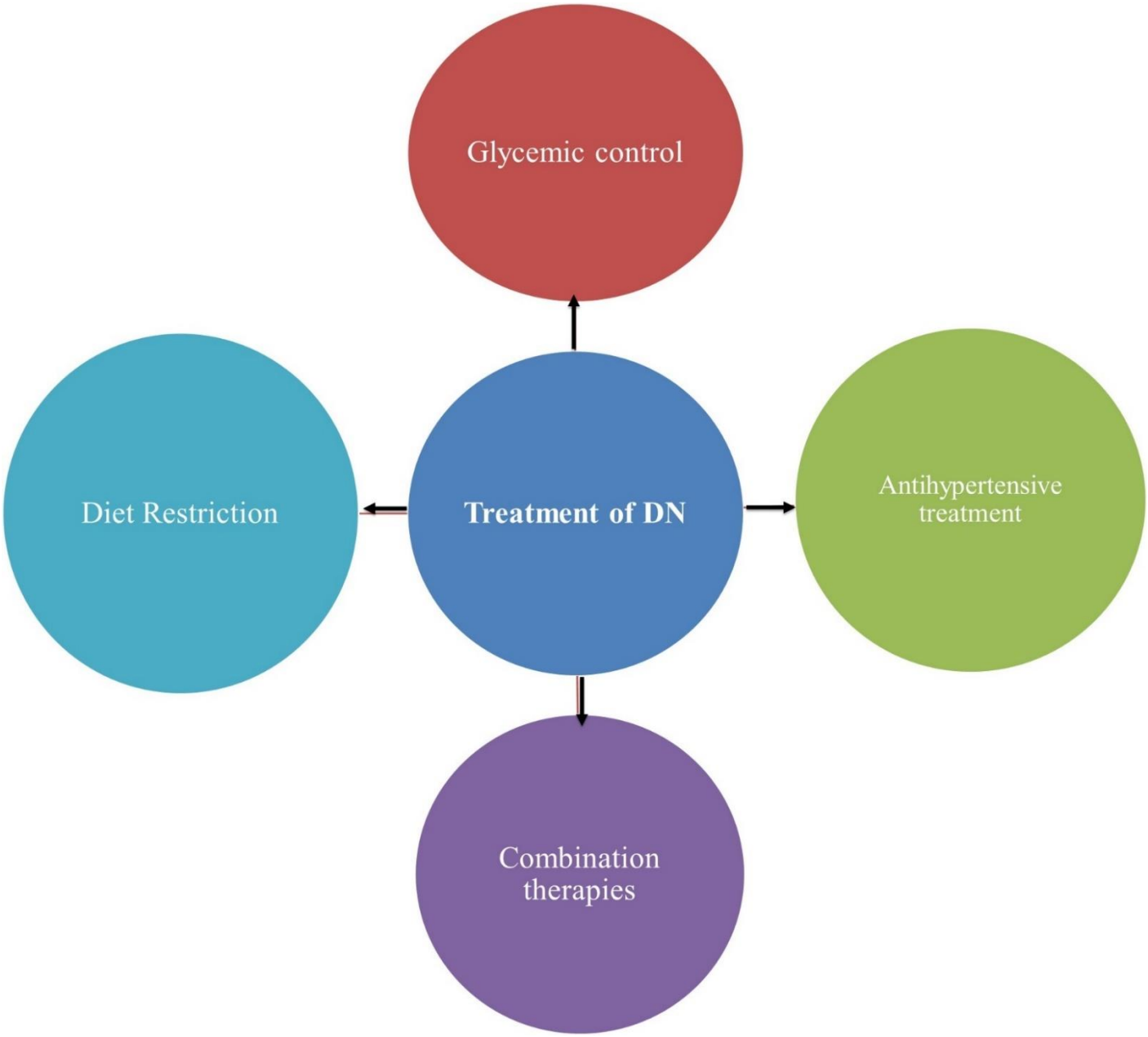


Figure 2.5: Current treatment methods employed for ameliorating DN

Glycemic control

It is already proven that DM causes the advancement of DN, in order to control DN, one should have strict glycemic control. Studies from clinical trials have proved that A1c levels >8% is a reason for DN in T2DM patients. Studies from DCCT 1995, have shown that strict glycemic control can delay the progression of DN by decreasing the rate of development of overt proteinuria and microalbuminuria. Strict glycemic control decreased the probability of microalbuminuria by 39% (DCCT 1995). Even after 7-8 years of DCCT, patients having strict glycemic control have reported a 40% reduction in microalbuminuria development (DCCT, 2003). A study held in the UK stated a 30% risk reduction in patients with DM having strict glycemic control (UKPDS, 1998). In another study, strict glycemic control delayed micro and macroalbuminuria (Wake, 2000). Rosiglitazone (antihyperglycemic drug) when compared with glyburide showed a decrease in UAE in DM patients thus proving its effectiveness in preventing DN (Bakris et al., 2003).

Blood pressure control

About 70% of DM patients have a high blood pressure of > 140/90 mm Hg (Whelton et al., 2018). There was a 50% reduction in cardiovascular events of diabetes when blood pressure decreased from 85 to 81 mm Hg (Hansson et al., 1998). Mogensen is credited to show that antihypertensive drug treatment can ameliorate renal injuries in patients of DM and DN (Mogensen, 1976). ACE inhibitors alleviate the risk of DN (Lindholm et al., 2002). In a study done by the Collaborative Study Group, patients with DM having proteinuria, were given a low/high dosage of ACE inhibitors and Ramipril showed a difference of 7mm Hg blood pressure. The group having a high dose of ACE inhibitors had lower blood pressure and a decreased rate of albuminuria (Bakris et al., 2003). Patients with high blood pressure but having a low risk of albuminuria can be treated with diuretics (Trujillo et al., 2021). RAS blockade with ACE inhibitors helps to ameliorate renal injuries by decreasing intraglomerular pressure (Viberti and Wheeldone, 2002 and Mogensen, 2003).

Diet restriction

In DM patients, protein diet restriction also helps in ameliorating DN. In a meta-study, restriction on protein intake (0.5-0.85 g /kg/ day) showed a beneficial effect on creatinine clearance, albuminuria, and GFR (Mitchell et al., 2020). T1DM patients exhibited a reduced risk of ESRD. The LDL cholesterol level should be < 100mg/dl for T2DM diabetic patients (Khan et al., 2019). Study states GFR may be preserved and proteinuria may be decreased by antilipidemic agents that degrade lipids (Fried et al., 2001). There was a 25% decrease in GFR events when 40mg of simvastatin was given to DM patients (Heart protection study, 2003). Anaemia also contributes to the progression of DN (Ito et al., 2021). If the haemoglobin (Hb) levels are <11 then hematopoietic treatment should be started (Laville., 2004). Patients with DN may have anaemia before renal failure due to the deficiency of erythropoietin (Bosman et al., 2001).

New therapies

The treatments described above may not be as effective, hence, potential new therapeutic approaches are needed. Benfotiamine derivative of thiamine had been shown to reduce the development of microalbuminuria by diminishing the activation of PKC, ROS, and AGEs (Babaei et al., 2003). Alagebrium (ALT-711) breaks the cross-linking of AGEs and reduces renal lesions, UAE, as well as blood pressure (Forbes et al., 2003). Ruboxisaturin, a PKC inhibitor is reported to decrease albumin excretion and normalized GFR in vivo models of diabetes (Kelly et al., 2003). The administration of modified heparin glycosaminoglycan to rat models having glomerulosclerosis ceased tubular matrix accumulation, prevented albuminuria, and overexpression of TGF-β1 m-RNA (Ceol et al., 2000). Treatment with a glycosaminoglycan, Sulodexide reduced micro or macroalbuminuria in diabetic patients (Gambaro et al., 2002). Second-generation inhibitor of AGEs: Pimagedine decreased GFR and protein in the urine (Bolton et al., 2004). Mycophenolate mofetil, an anti-inflammatory immunosuppressive drug reduced glomerular injury (Utimura et al., 2003). Administration of Infliximaba known chimeric anti-TNF-α antibody to diabetic rats reduced albuminuria and the presence of TNF-α in the urine. Pentoxifylline (PTF) is a phosphodiesterase inhibitor derived from methylxanthine and can significantly lower the levels of mRNA encoding TNF-α and also modulate IFN, IL-1 and IL-6 and has been used to improve blood flow by reducing its viscosity (Cooper., 2004 and Moriwaki et al., 2007).

Phytochemical-based approach to fight DN

Many phytochemicals have been reported in the literature that has been proven effective in treating diabetes. They treat diabetes in many ways some ameliorate diabetes, some have anti-diabetic activity, and some exert hypoglycemic effects (Govindappa, 2015). Some phytochemicals from medicinal plants also help in reducing DN; a few of them are (Khazim et al., 2013), *Curcuma longa* (Jeenger et al., 2015), berberine from *Berberis vulgaris* and andrographolide from *Andrographis paniculata*. These medicinal plants not only ameliorate renal injury but also control different ways of signaling (Figure 3). The antioxidants present in them provide protection to nephrons by decreasing oxidative pressure that ultimately controls diabetic complications. A few examples of medicinal plant extracts and their bioactive compounds are discussed below (Table 2.2)

Table 2.2: Phytochemicals in ameliorating DN

S.No	Plant Name	Phytochemical	Result	Mode of Action	Reference
1.	*Abroma augusta*	Flavonoids and phenolics	Reduces vascular inflammation, hyperlipidemia, hyperglycemia,	Downregulate the expression of Interleukins	Khanra et al., 2015

			and oxidative stress.		
2.	*Camellia sinensis*	Catechin	Preventing oxidative stress, hypertension.	Inhibits the formation of AGEs	Hao et al., 2012
3.	*Eugenia jambolana*	Seed extracts	Ameliorate diabetic nephropathy	Improving kidney weight, BUN (blood urea nitrogen), creatinine, uric acid, and urine.	Esther and Manonmani, 2014
4.	*Glycine max*	Anthocyanin	Delay progression of DN and protects kidney from renal injuries	Reversing renal damage and reduce oxidative stress and apoptosis by activating AMPK.	Kusirisin et al., 2009
5.	*Myrciaria cauliflora*is	Polyphenols and anthocyanins	Ameliorate DN	Inhibiting RAS/P13K pathway	Wu et al., 2016
6.	*Polygonatum odoratum*	Homoisoflavanones	Provide protection against nephropathy	Inhibitory action against AGE	Dong et al., 2010
7.	*Salvia miltiorrhiza*		provide protection against DN	Suppress the expression of CTGF	Peng et al., 2005
8.	*Silybum adans*		Attenuate DN	Increase the activity of glutathione	Rafieian-Kopaie and Nasri, 2012

				peroxidase, catalase	
9.	*Vitis vinifera*	Resveratrol	Reduce renal dysfunction	Upregulate activation of AMPK.	Nassiri-Asl and Hosseinzadeh, 2009
10.	*Cinnamomum cassia*	Cinnamicaldehyde	Ameliorate metabolic disorders	Protect from renal damage	Zheng et al., 2011
11.		Cinnacasside, 15-hydroxy-t-muurolol, Cinnamoid E and 10α-Hydroxyaromadendrane	Provide protection against DN	Impaired FN in hyperglycemia	Yan et al., 2015
12.	*Cordyceps cicadae*	Ergoestrol	Delays DN	Improve renal fibroblast proliferation through TGF-β1	Hsu et al., 2015
13.	*Ophiopogon japonica*		Treat DN	Inhibits NF-κB expression through anti-fibrotic and anti-inflammatory activity.	Lu et al., 2014
14.	*Tanacetum parthenium*	Parthenolide	Prevent DN	Downregulates NFκB-binding protein (IκBα) under high glucose conditions.	Xu et al., 2013
15.	*Withania coagulans*		Reduction in renal injury	Restore glutathione levels, inhibiting lipid peroxidation, and reducing the levels of	Ojha et al., 2014

				proinflammatory cytokines and hyperglycemia.	
16.	*Berberis vulgaris*	Berberine	Ameliorate DN	Activates AMPK and suppress oxidative stress, inhibits iNOS, and EMT signaling pathways.	Jyothilakshmi et al., 2013
17.	*Beta vulgaris*	Betanin	Protection against DN	Reduce glomerulosclerosis, glomerular surface area, and tubulointerstitial fibrosis.	Sutariya and Saraf, 2017

Abroma augusta has been extensively used to treat diabetes by reducing vascular inflammation, hyper-lipidemia, hyperglycemia, and oxidative stress by downregulating the expression of IL-1β and TNF-α (Khanra et al., 2015) suggesting its capability as a potent nephroprotective agent in DN. *Benincasa cerifera* also known as Kusmanda is known to provide protection against renal failure and prevents LPO (lipid peroxidation) which is mainly due to oxidative stress (lipid peroxidation). *Camellia sinensis* commonly called green tea has major implications in preventing oxidative stress, hypertension, and diabetes. A study conducted on STZ (streptozotocin) induced rats having hypertension demonstrated that green tea treatment reduced renal injury (Hao et al., 2012). *C. sinensis* was reported to be rich in polyphenols like epigallocatechin, epicatechin gallate, and epicatechin. The *C. sinensis* is known to ameliorate diabetic conditions due to these phenols that inhibit the formation of AGEs, IL-1b, and TNFα. Catechin is a bioactive compound majorly responsible for preventing diabetes and its complications (Zhu et al., 2014). *Curcuma longa* also called turmeric has a bioactive constituent called curcumin. Curcumin is well-evaluated and shows nephroprotective effects attributed to its antioxidant property (Hao et al., 2012 and AL Tamimi et al., 2021). *Eugenia jambolana,* the common name being Jamboo has been used in treating diabetes even before insulin was discovered. In rats having DN, its seed extracts resulted in improvement in kidney mass, BUN (blood urea nitrogen), creatinine, and uric acid.

Glycine max also called soybean, is known to delay the advancement of DN and protect the kidney from renal injuries. Recently, the effect of the extract of *G. max* rich in anthocyanin was elucidated for renal lipotoxicity in DN. Results are indicative of its capacity to reverse renal damage and reduce oxidative stress and apoptosis

by activating AMPK (Kusirisin et al., 2009). *Linumusitatis simum* also referred to as linseed or flax was also found protective in DN. Experiments were performed on obese rats where substituting a meal with flax seeds reduced glomerular and tubular interstitial lesions and proteinuria. It is more effective than soybean in reducing proteinuria indicating the fact that a decrease in proteinuria is independent of glycemic control or protein intake (Mani et al., 2011). The peel extract of *Myrciaria cauliflora* is (rich in polyphenols and anthocyanins) inhibited the PKB pathway (Wu et al., 2016). *Polygonatum odoratum* potency is due to the homo-iso-flavanones present in it that are known to show inhibitory action against AGE inhibition and provide protection against nephropathy (Dong et al., 2010). *Salvia miltiorrhiza* provides protection against DN by suppressing the expression of CTGF, fibronectin, PAI, and TGF-β1 in the renal cortex (Peng et al., 2005). *Silybum adans* commonly called milk thistle was found to increase catalase, glutathione peroxidase, and LPO in kidney tissues and attenuate DN (Rafieian-Kopaie and Nasri, 2012).

Terminalia chebula is typically called the "King of medicine". It has been traditionally used in treating diarrheas, vomiting, diabetes, and asthma by exerting antioxidant activity and providing protection against renal injuries. The evidence supports that it is a potent candidate to give protection against DN (Sabu and Kuttan, 2002). Other studies on the mechanism of action of resveratrol revealed that it is capable of suppressing inflammation induced by diabetes and halts the RMCs proliferation by PKB/NF-κB signaling pathways. *Zingiber officinale* commonly known as ginger has 6-gingerol as its major component. It induces a hypoglycemic effect by blocking the serotonin receptor (Al-Amin et al., 2006). *Vitis vinifera* has resveratrol as one of its bioactive compounds that are known to upregulate AMPK and reduce renal dysfunction (Nassiri-Asl and Hosseinzadeh, 2009). *Andrographis paniculata* has flavonoids, polyphenols, and diterpenoids as its major constituents. An experiment on STZ and high fructose diet-fed rats showed its hyperlipidemic and hypoglycemic effects (Rao, 2006 and Okhuarobo et al., 2014). *Cinnamomum cassia* has cinnamic aldehyde as its major volatile compound. It is known to ameliorate metabolic disorders in case of renal damage by masking NRF2 activation. Components like cinnacasside, 15 hydroxy-t-murolol, cinnamoid E, and α-Hydroxyaromadendrane provide protection against DN by impairing MCP-1 and fibronectin expression under hyperglycemic conditions (Yan et al., 2015). Ergosterol from *Cordyceps cicadae* controls renal fibrosis progression by improving renal fibroblast proliferation through TGF-β1 (Hsu et al., 2015 and Yu et al., 2016). *Ginkgo biloba* has been reported to show neuroprotection against glomerulosclerosis (Tang et al., 2014). *Ophiopogon japonica is* known to treat DN by inhibiting NF-κB expression through anti-fibrotic and anti-inflammatory activity (Lu et al., 2014). Parthenolide which is a sesquiterpene lactone obtained from *Tanacetum parthenium* has known to enhance the expression and activation of NF-κB and mesangial cells, and downregulate NFκB-binding protein (IκBα) under high glucose conditions hence showing the ability to prevent DN (Xu et al., 2013). *Berberis vulgaris* has berberine as its major phytochemical. Berberine has the potential to ameliorate DN by activating AMPK and suppressing oxidative stress, inhibiting iNOS, NF-κB, TNF-α, and EMT signaling pathways (Zhang et al., 2016). *Withania coagulans* target inflammatory and immunoregulatory cytokines in DKD and its extract is known to work in many ways restoring glutathione levels, inhibiting lipid

peroxidation, reducing the levels of proinflammatory cytokines and hyperglycemia, suggesting amelioration of DN (Ojha et al., 2014).

Betanin a chromoalkaloid of beetroot had been found to reduce glomerulosclerosis, glomerular surface area, and tubulointerstitial fibrosis and is thus protective against streptozotocin-induced diabetic nephropathy. Trigonelline was found to be potent enough to alleviate STZ-induced kidney damage. Trigonelline treatment significantly lowers the serum levels of urea and creatinine in diabetic rats. It decreased TNF-α levels and improves the antioxidant enzyme activities and GFR as compared to diabetic rats (Ghule et al., 2012). However, the protective role of natural products like phytochemicals in removing GM via modulating epigenetics is still unexplored. Much work is needed with both *in vitro* and *in vivo* animal models to understand the basic mechanisms, and to specify the roles of plant phytochemicals in preventing and curing diabetic complications. Such new knowledge will help to design effective therapeutic strategies for treating metabolic abnormalities.

Phytomolecule enriched in Broccoli, sulforaphane, is known to inhibit the expression of DNMT1, HDACs and reduce promoter methylation (Traka et al., 2005 and Meeran et al., 2010). Garlic has organosulfur compounds that are known to induce the acetylation of histones and protect against several anomalies (Druesne et al., 2004). Tomatoes are rich in lycopene, which is believed to give relief from OFRs (Valero, et al., 2011). It works by altering gene methylation (Batoon et al., 2008). Quercetin is another important phytochemical involved in ameliorating DN by acting as a DNMT1 inhibitor. It works by repressing the TNF and promotes cell apoptosis by acetylation of H3 (Lee et al., 2011). In several reports, it was shown that quercetin, by MAPK insulin-dependent mechanism, is known to stimulate glucose uptake (Chen et al., 2016).

Studies done on the effects of nutrition and chemicals like bisphenol A, phthalates, mercury, and lead on gene-environment interactions (epigenetic/genetic regulations) using clinical samples, animal models, and human studies. It was demonstrated in Agouti mice that maternal genistein supplementation (250 mg kg−1) provides protection against obesity in off-springs (Dolinoy et al., 2006). Luteolin and fisetin (polyphenols found in fruits and vegetables) protect via inhibiting hyperglycemia-induced HAc and cytokine production in monocytes with NF-κB activation (Kim and Yun, 2014). (-)-Epicatechin (EC), a cocoa flavonol also displays anti-diabetic effects by decreasing hyperglycemia-induced histone deacetylase (HDAC 4) activity and increasing Histone 3 lysine9 (H3K9) acetylation in human monocytes (Cordero-Herrera et al., 2017). Esculetin was found to ameliorate DN by regulating various histone modifications in rats (Kadakol et al., 2015). The ability of these phytochemicals can neutralize diabetes-induced epigenetic modifications puts them under question for their possible beneficial effects in improving GM syndrome associated with diabetes and in preventing the occurrence of DN. There is a high possibility to observe the beneficial effects of these phytochemicals in removing GM and diabetes progression.

In the present study, we aim to identify and characterize such bioactive compounds. There is a multitude of reports which had evaluated the beneficial effects of many plants but mostly are done on crude extracts. The

crude extract is generally a mixture of many phytochemicals in variable proportions. One cannot ascertain which biological activity is the result of the presence of which phytochemical. So, there is a need for systematic screening of therapeutically important bioactive compounds present in plants for the drug discovery process. The phytochemicals with epigenetic modulatory effects can possess the potential to remove GM and thus be preventive in associated complications. In short, the need of the hour is to screen out phytochemicals that can be effective in treating disease conditions associated with diabetes like glycemic or GM and diabetic nephropathy.

CHAPTER 3

HYPOTHESIS

The pathogenesis of DN consists of sophisticated interactions between hemodynamic and metabolic factors, the major role played by AGEs, TGF-β, and Ang II. Multitudes of reports have supported the evidence for the role of epigenetic mechanisms like DNAme, histone PTMs, and miRNAs in the progression of DN. GM is one of the factors for persisting epigenetic modifications. Many reports have shown the role of inflammatory and fibrotic genes but there are many to be revealed. Since epigenetic changes are reversible there are plethoras of opportunities for drug development based on combination therapies with antagomirs for DN. It is expected that the emerging field of epigenetics can unravel the path for new biomarkers so that the detection and treatment of GM and DN could be made easier. The complex nature of kidney and DN progression limit the use of synthetic drugs because of their side effects. So, the need of the hour is to develop a more efficacious drug with the most negligible side effects. Many already published supports the fact that phytochemicals that are potent nephroprotective agents can be used for treating DN. They are also the natural regulators of epigenetic mechanisms and thus possess the potential to erase or reverse these reversible events. As aberrant epigenetic marks are a major role player in GM-causing diabetes and DN, these phytochemicals might also be protective in erasing GM and protecting against metabolic diseases.

Therefore, we hypothesize that phytochemicals possess the potential to reverse epigenetic alterations, can erase GM, and are thus protective against metabolic diseases like diabetic nephropathy. To fish these phytochemicals, we ulternative hypothesis that "**similar structures will have similar biological activities**". Null hypothesis (H_o= similar structures will not have similar biological activities). An in-silico similarity searching technique was initially utilized using the structures of known epigenetic modifiers to fish out the bioactive chemical that can prevent GM and diabetic nephropathy.

Chapter 4

Aims and Objectives

Aims

Synthetic drugs are used to treat DN, but they are loaded with side effects. So, we need to look for alternatives with no side effects. We seek to identify such naturally occurring epigenetic modifiers that are structurally related to the already present medicines, then test their binding affinity and molecular interaction with SIRT-1 in silico, investigate them in reducing oxidative stress and the associated epigenetic changes, and further validate our findings with the help of in vitro (cell culture) as well as in vivo (SD rats) models. The following objectives were created in order to accomplish the study's goals listed below. The following objectives were achieved by combinatorial in silico, in vitro, and in vivo approach. In in silico approach, a mix of structural similarity searching along with molecular docking followed by ADME studies were used to fish out potent natural epigenetic modifiers. These natural epigenetic modifiers were assessed for their ability to modulate antioxidant activity as ROS play a major role in the pathogenesis of DN and GM. To validate the results we mimicked the diseased condition in vivo as well, where we evaluated the lipid profile, kidney and liver function test, extent of kidney damage, and any aberration in histone acetylation levels. We checked whether the chosen phytochemicals were able to ameliorate these parameters or not.

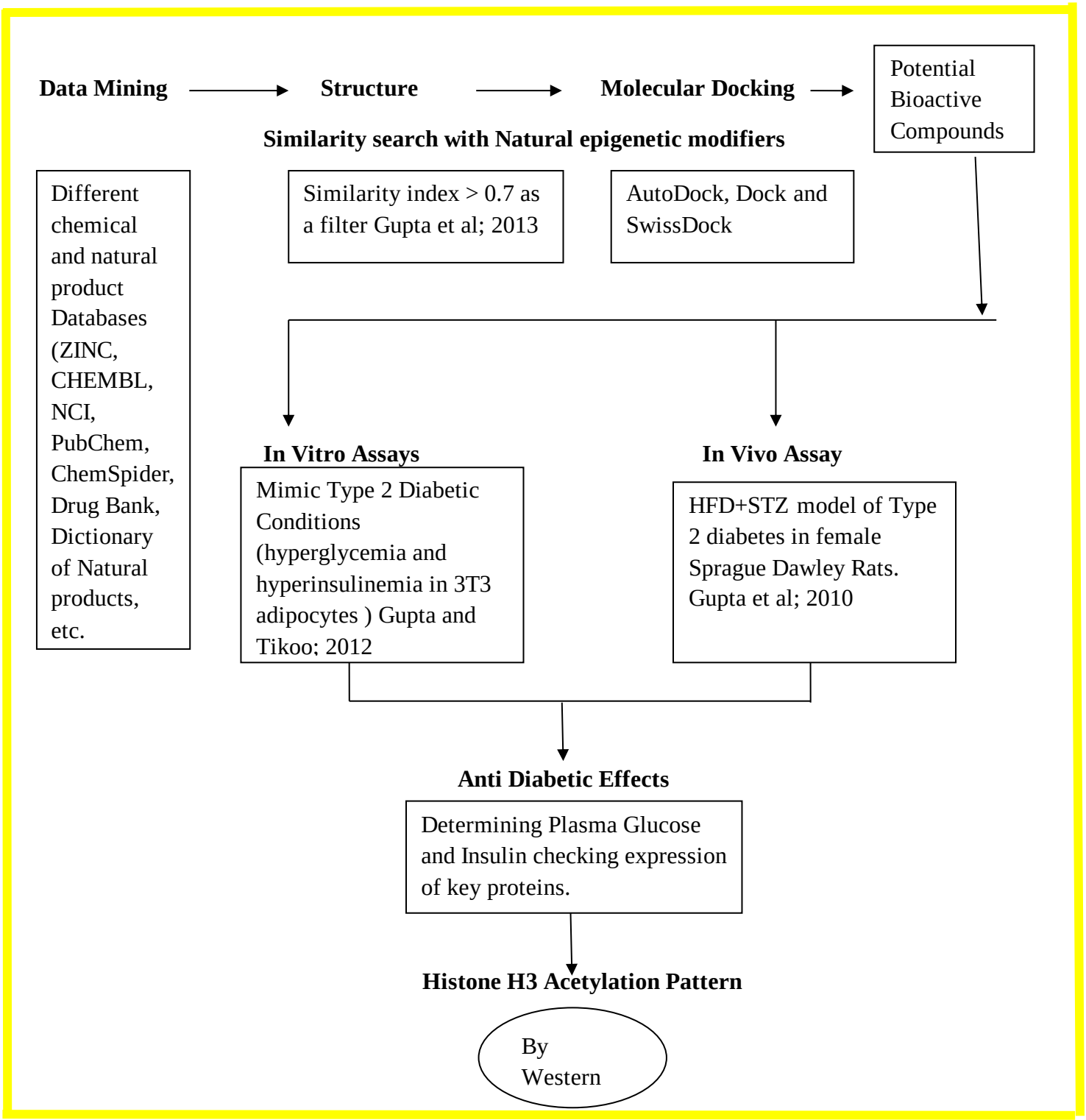
Objectives

1. Structural similarity searching of known epigenetic modifiers to screen promising novel phytochemicals by molecular docking studies
2. To establish a successful in vitro and in vivo model of diabetes-associated glycemic memory (GM) and evaluate the beneficial effects of potential phytochemicals
3. To evaluate the histone acetylation modifying potential of selected bioactive components and removing GM

Chapter 5

Materials and Methods

Research Design:



5.1 Material, Method, and Experimentation: All the study has been carried out at Lovely Professional University

5.1.1 Chemicals and Reagents

The analytical grade chemicals used in the analysis are UMBelliferone (99%pure), gamma-aminobutyric acid (99% pure) and metformin was purchased from Sigma-Aldrich chemicals, cholesterol was purchased from Hi Media Lab Mumbai, casein was purchased from Saras Casein (Orai), vitamin and mineral mix was purchased from a local vendor, Carboxyl methyl cellulose, DL-Methionine, and yeast powder was procured either from S.D Fine Chemicals or HiMedia India Ltd.

5.2 In silico studies

5.2.1 In silico data compilation and similarity searching

- Different chemical and natural product databases like ZINC, ChEMBL, PubChem, ChemSpider, and Drug Bank were searched to collect information regarding 2D and 3D structures, SMILE notation, experimental data on different biological activities, and physicochemical properties.

- Each structure of the phytochemicals was used as a query to search the chemical/natural databases by using the similarity index > 0.7 as a filter.

- Checked the biological activities of similar molecules reported or given in the databases or literature.

5.2.2 Molecular Docking Studies

A technique for anticipating the query structure's binding conformations at a protein's active site is called molecular docking (Grosdidier et al., 2011 and Ferreira et al., 2015). The 3D structure of the human SIRT1 protein was taken from the PDB. Following a thorough assessment of the literature, we selected phytochemicals that were promising candidates. We used the web-based docking service Auto Dock -Vina (Trott & Olson, 2010) to identify the SIRT1's most likely active ligands. Protein Data Bank (PDB) was used to find the SIRT1 protein structure (https://www.rcsb.org/). In order to compare these similar structures, we used the same parameters. Here, we used PDB ID:4I5I for docking. Following the protocol given by Bashary & Khatik, 2019, ligand and receptor preparations for the molecular docking investigations were done.

5.2.2.1 Preparation of Receptor

We opened the protein 4I5I.pdb file for editing in the auto dock edit window after downloading it in PDB format from the PDB database. The protein was repaired, with missing atoms added, water molecules removed, and polar hydrogens added, as the protein structure showed on the screen. Only cofactors that are normally bound to the protein chain were retained, and the native ligand was also removed from the active site. The protein was saved in pdbqt format after all the modifications were completed.

5.2.2.2 Ligand preparation

The ligands were retrieved as sdf files from the PubChem database and then transformed with Software Open Babel into pdbqt format. Using ChemBio Office software, the energy of the ligand was minimized. By selecting the input molecule in the edit panel, ligands were uploaded in the PDB file to the AutoDock-vina. Non-polar hydrogens along with gastegier charges were assigned to each ligand. The generated ligands were now saved in the pdbqt format in the final stage, which also involved selecting the torsion tree and detecting the root.

5.2.2.3 Determining the active site of receptor and visualization of docking interaction

Knowing the binding cavity in which our ligand will bind is crucial before docking. Python molecule viewer (PMV) was used to prepare the receptor 4I5I and identify its active site. The native ligand was put into our receptor 4I5I in PMV in pdb format, and the binding site was examined. Every native ligand cocrystallized with the receptor has a cavity for the binding site. After identifying the active site cavity, we used the same active site cavity in the Auto Dock vina configuration file by using its X, Y, and Z attributes, which were -48.349560, -2.168960, and 78.207680 respectively. The ligand was loaded in pdbqt format and the map type was selected after finding the binding cavity and preparing the protein and ligand in pdbqt format for docking. The next step was to configure the grid box by selecting the option to center on the ligand in the panel. The space of 27000 Angstrom was used for the search. All of these settings resulted in the protein being saved in pdbqt format. The identical X, Y, and Z coordinates 48.349560, -2.168960, and 78.207680—were used to create the configuration file. To analyze the outcomes, the docking procedure was conducted using a command line. The output file for the command prompt generated the docking scores as binding affinity (kcal/mol) values. Conformation postures were seen in the docking data and the permitted Root Mean Square Deviation (RMSD) was 1.5. If the RMSD value is less than 2.0 and more than 80% of the residues are located in the most desired location, the simulated structure can be considered good (Selvaraj et al., 2014).

5.2.3 ADME Studies

In silico ADME (Adsorption, distribution, metabolism, and excretion) characteristics of substances were predicted using the SwissADME tool (Daina et al., 2017). By linking a compound's water solubility and lipophilicity, the in silico ADME characteristics were expected to assess its toxicity. The pharmacokinetics, physiochemical characteristics, water solubility, and lipophilicity of the drug were determined using Swiss ADME (http://www.swissadme.ch/index.php). In silico models have been employed extensively where chemical structures are numerous but resources and availability of those are problems, as an alternative for experimental procedures to predict ADME (Dahlin et al., 2015).

For a potent molecule to be effective as a drug, it has to reach its target site where it should remain in its bioactive form long enough for the events to occur. Development of drugs involves ADME assessment i.e, absorption, distribution, metabolism, and excretion in a stage where a number of compounds are numerous whereas the availability of physical samples is limited. To overcome this challenge in silico computer models, act as an

alternative to this. We have here used the SwissADME web tool which is a predictive model for pharmacokinetics, drug-likeness, and physicochemical properties. In this, BOILEDegg is one important method that shows whether your compound is being GI absorbed or is BBB permeant or not. A spider web graph is a unique way of showing data across many dimensions. We did ADME studies on a few similar phytochemicals for each parent compound.

To do the ADME studies canonical SMILES were retrieved from the ChEMBL database and used in the SwissADME database.

5.3 In vitro studies

5.3.1 DPPH Free Radical Scavenging Activity

The method developed by Sharma et al., 2009 was used to assess the activity of DPPH (1,1 diphenyl-2-picrylhydrazyl (DPPH) in scavenging free radicals A sample mixture with a total DPPH radical concentration of 0.1 mM received UMB and GABA at various concentrations. Before being allowed to stand in the dark for 60 minutes, the solution was vigorously stirred for 1 minute. The scavenging capability was read at 517 nm where we used ascorbic acid as the positive control. The sample concentration was plotted against the percentage of inhibition in the reaction system.

5.3.2 Cell line toxicity test

The MTT test was used to visualize cell cytotoxicity, and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or (MTT) Assays allow us to quantify cell death in cell culture, as stated by Kumar et al., 2018. In preclinical drug development, potentially therapeutic compounds are frequently assessed on cell lines to determine any potential harmful effects on the cells.

5.3.2.1 Principle

The MTT assay is used to assess cell viability, proliferation, and cytotoxicity by measuring cellular metabolic activity. This colorimetric assay is based on metabolically active cells reducing a yellow tetrazolium salt MTT to purple formazan crystals (Vistica et al., 1991). NAD(P)H-dependent oxidoreductase enzymes in live cells convert MTT to formazan. The insoluble formazan crystals are dissolved in a solubilization solution, and the resulting-colored solution is quantified using a multi-well spectrophotometer to measure absorbance at 540nm. A darker color solution implies more active cells and hence increased metabolic activity.

5.3.2.2 Reagents

MTT

DMSO

5.3.2.3 Procedure

- Prior to adhering for 24 hours in a CO_2 incubator at 37 °C, cells were first added in 96-well plates with a density of 1 x 10^8 cells per well.
- After 24 hours of incubation, a new medium was added to the culture media.
- Cells were then exposed to various concentrations of the test drug for 24 hours at 37 °C in a CO2 incubator, after which the old medium was changed out for the new.
- A working solution of MTT 10 mg/ml was added to the well, and 96-well plates were then incubated for 4 hours at 37 °C in a CO2 incubator.
- The medium was removed, and the formed formazan crystals were dissolved in DMSO per well for 30 minutes at 37 °C in a CO2 incubator.
- Finally, the intensity of the formazan crystals (purple color) was quantified using an ELISA plate reader set at 540 nm.
- The percentage of viable cells in treated and untreated cells was used to represent cell viability since there was a positive correlation between the number of viable cells and the absorbance at 540 nm.

5.3.2.4 In vitro induction of diabetes in 3T3L-1 cell line

5.3.2.4.1 Material

- DMEM
- Mannitol
- Glucose
- Antibiotic/AB
- Fetal bovine serum/ FBS
- Phosphate buffered saline/ PBS
- Distilled water/DW

5.3.2.5 Procedure

- The NCCS, Pune cell bank provided the 3T3L-1 cell line.
- The cells were cultured in the proper concentration of Dulbecco's Modified Eagle Medium (DMEM) (either low/normal glucose (5 mM + 20 mM mannitol, for osmotic balance) or high glucose (25 mM), per the usual procedure and standard protocol.
- The cells were given fetal bovine serum (10%) and antibiotics (Penicillin 100 IU/ml/Streptomycin 100 mg/ml) under 5% CO2 at 37°C. After achieving 80–90% confluency, the cells were halted in the G0 phase. This mimic the symptoms of hyperinsulinemia and hyperglycemia.
- The procedure was completed in triplicates.
- **In Vitro model establishment (in 6 well plates)**

- **Low glucose plate** seeded cells in a 6-well plate in 5mmol of glucose, after achieving 70% confluency given respective treatments to the group.
- **High glucose plate** seed cells in a 6-well plate in 30mmol of glucose, after achieving 70% confluency given respective treatments to the group (Figure 5.2).
- **Diet reversal plate** first cells were grown in high glucose medium and then while plating in a 6-wells plate switched media to low glucose, after achieving 70% confluency given respective treatments to the group (figure 5.1).

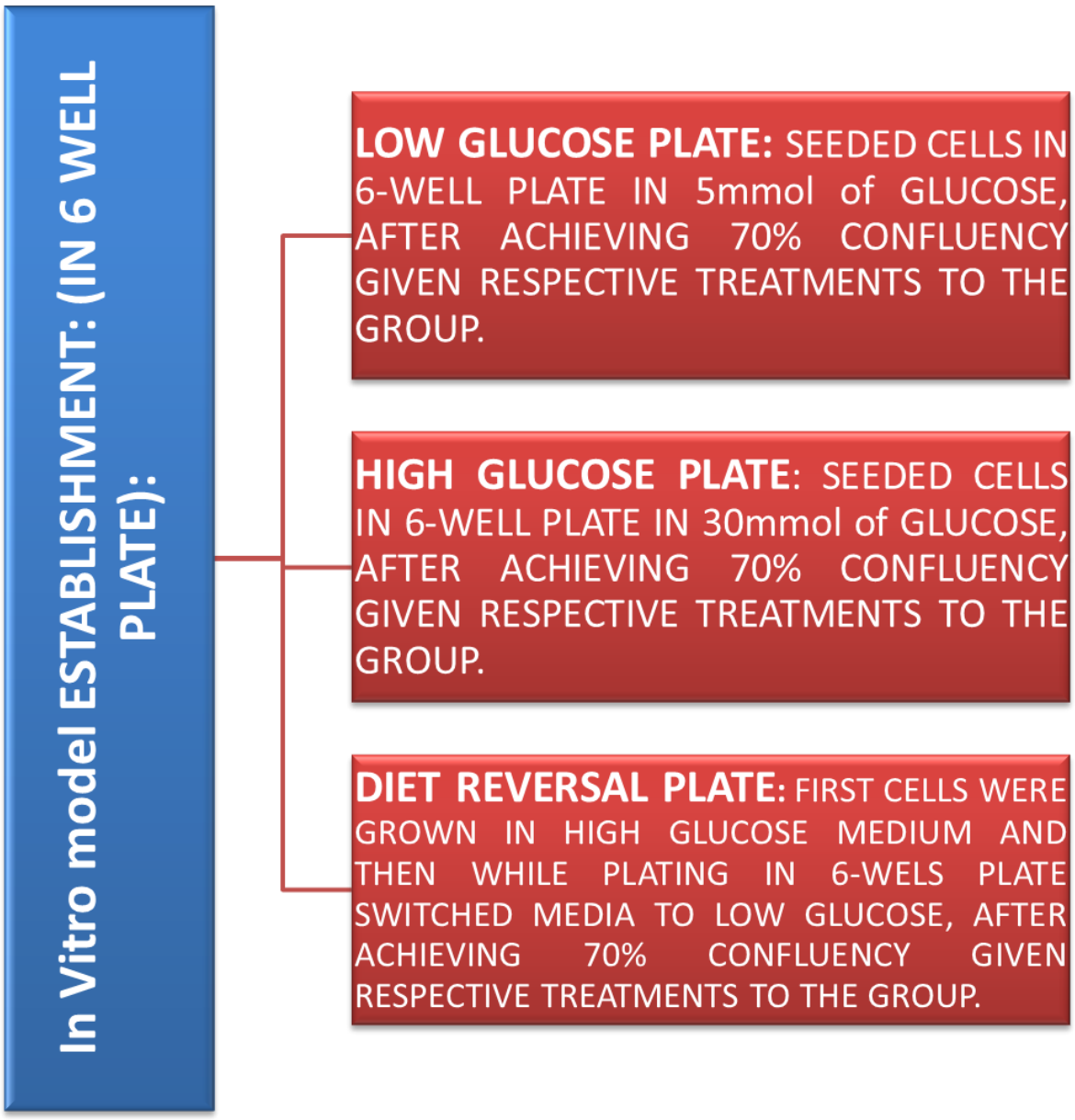


Figure 5.1: In vitro GM model development using 6 well plates of low and high glucose DMEM media and diet reversal.

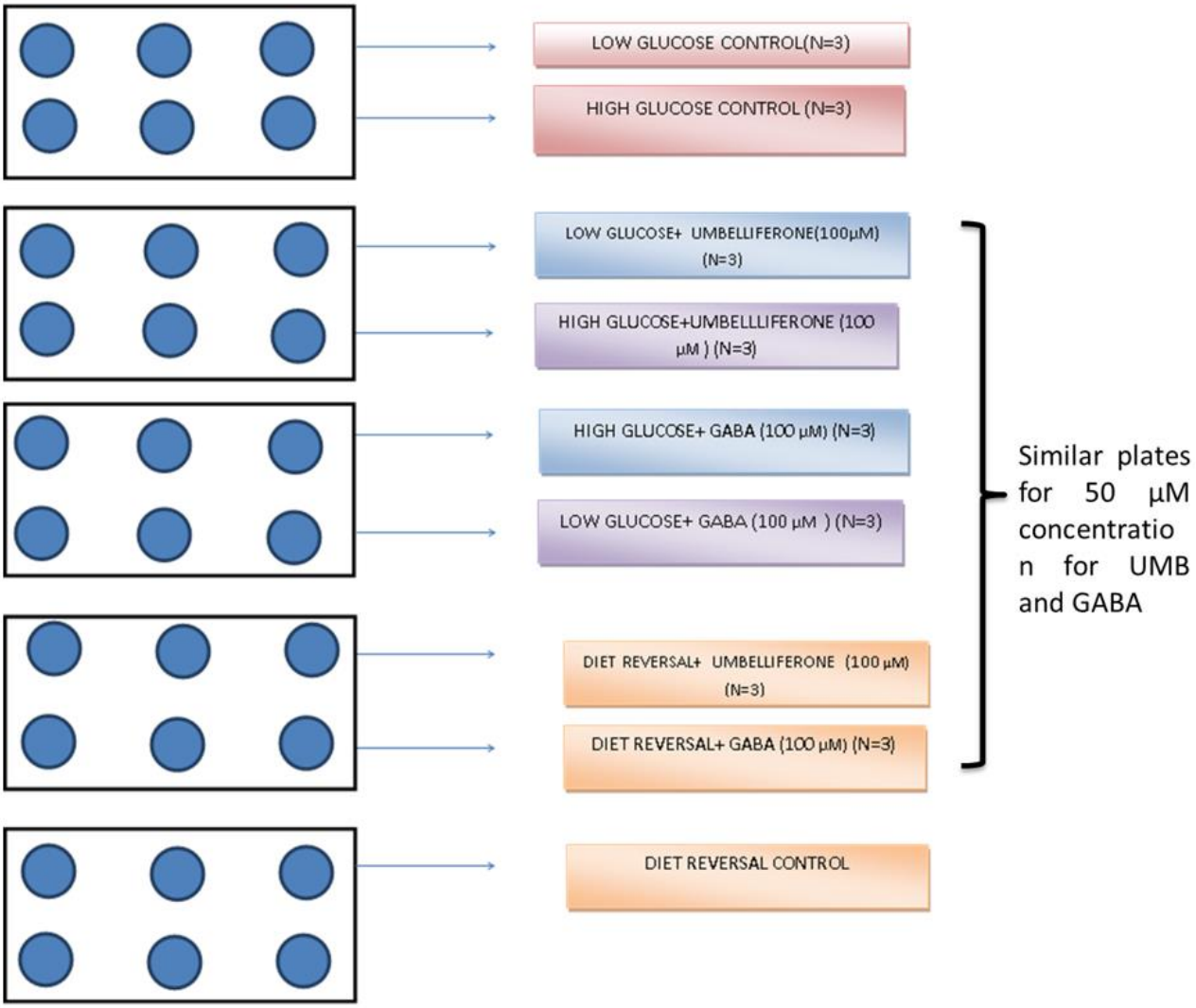


Figure 5.2: Cell culture study schematics for developing GM

5.3.3 Cell lysis and preparation of cell lysate for protein array (6-well format)

- We added protease and phosphate inhibitors to the lysis solution in order to prevent the breakdown and modification of isolated proteins and to get the highest protein yield during cell lysis.
- Following the experimentally designed treatment of cells on a 6-well plate, the cells were rinsed with ice-cold phosphate buffer saline (PBS).
- To maintain levels of HAc, sodium butyrate (5 mm) was added to the lysis solution.
- Then, a lysis buffer was added to the plate. The lysate was then collected in microcentrifuge tubes after the cells were scraped off using a cell scraper. The cell lysate was then centrifuged in a cold microcentrifuge for 7 minutes at 300g (1700rpm).
- We kept the supernatant and threw out the pellet.

5.3.3.1 Preparation of tissue/cell homogenate

We utilized a Dounce homogenizer to make the homogenate using Ethylenediamine tetra acetic acid (EDTA) (0.1M) - Tris Chloride (0.1M) buffer, which was made by combining both solutions and setting pH

7.4. Beta-Mercaptoethanol has added to the buffer right away before the cells and tissues were homogenized. Always keep the homogenizing tube in an ice-filled beaker. A motor-driven Teflon pestle rotating at 3000 revolutions per minute was used to homogenize the cells. The produced homogenate was then filtered through cotton gauze and kept in plastic vials with the proper labels in a refrigerator (-18^{o}C) until it was needed again. This 10% homogenate (1g cells in 9 ml buffer) was used as a starting homogenate from which appropriate dilutions with the appropriate buffers were carried out whenever necessary to obtain the working homogenate fraction.

5.3.3.2 Post-nuclear supernatant preparation (PNS)

The cell homogenate was spun in a C-24 REMI cold centrifuge at 4400g (10000 rpm) for 5 minutes at 4°C to get the nuclear pellet and supernatant. For subsequent usage, the PNS was collected and stored at -18 degrees Celsius.

5.3.3.3 Post mitochondrial supernatant (PMS)

The supernatant was centrifuged for 10 minutes at 4400g (10,000 rpm) at 4°C after the nuclear pellet had been removed in order to get the mitochondrial pellet and post-mitochondrial supernatant. The PMS was held at -18 C for several estimates.

5.4 Estimating protein

According to the standardized procedure outlined by (Lowry et al.,1951), protein content was assessed using a standard curve constructed from bovine serum albumin (BSA).

5.4.1 Principle

The existence of the amino acids tyrosine and tryptophan is the foundation of this approach. Folin's reagent, which interacts with protein to produce a colored complex, was applied. The amino acids in the protein react with copper (alkaline) to diminish phosphomolybdate, which is the cause of color production. The amount of aromatic amino acids present controls the color's intensity.

Reagents

Reagent A = 2 percent sodium carbonate in 0.1N NaOH

Reagent B=1 percent $CuSO_4$ in Double distilled water.

Reagent C = 2 percent Sodium Potassium Tartrate in Double distilled water

Lowry’s Reagent= Reagent A+B+C in ration 49:0.5:0.5(v/v)

Dilute Folin’s Ciocalteau’s Reagent with DDW in a ratio of 1:1(v/v)

Dissolve 20mg of BSA in 1 ml of DDW= Stock protein standard

5.4.2 Procedure

Sample preparation in lowry's protein estimation

Reagents	Blank(ml)	Standard(ml)	Test(ml)
DDW	1	0.9	0.9
Standard	-	0.1	-
Test Sample	-	-	0.1
Lowry's Reagent	5 ml to each	5 ml to each	5 ml to each

Incubated tubes at 37°C for 15 min and then added 0.5 ml of Folin's Ciocalteau's Reagent. Then again incubated tubes for 30 min at 37°C. Absorbance was recorded at 670nm

5.5 Estimation of Catalase

It is measured in PNS according to the method of Luck (1971).

Principle

Catalase is an enzyme that decomposes H_2O_2 to H_2O

Reagents

- Phosphate Buffer (0.2M, pH=7.0)
- In 200ml of DDW add 1.79gm of $KH_2PO_{4=}$ Solution A
- In 300 ml of DDW add 3.52gm of $NaHPO_4.2H_2O$= Solution B
- Mix Solution A and B in a ratio of 3:7 (pH=7.0)
- In 100 ml of Phosphate buffer add 160µl of $H_2O_{2=}$Working phosphate buffer

Procedure

Table 5.1: Procedure for Catalase estimation

Reagents	**Blank**	**Test**
Sample	25 µl	25 µl
Phosphate buffer+ H_2O_2	-	3.0 ml
Phosphate buffer- H_2O_2	3.0 ml	-

O.D. was measured at 240nm for 2 minutes at the interval of 30 seconds.

Enzyme activity for catalase was calculated using the Molar extinction coefficient of **$43.6M^{-1}cm^{-1}$** was used and the result is expressed in µmoles of H_2O_2 decomposed/min/mg protein.

5.6 Estimation of GST

It is performed by the method by Habig et al, 1974 in PMS.

Principle

It is an enzyme that catalyzes the reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with the sulphydryl group of Glutathione.

The conjugate of CDNB-Glutathione absorbs at 340nm.

Reagents

- 100mM of Potassium phosphate buffer(pH=6.5)
- 1mM GSH (reduced glutathione) (pH=6.5)
- Dissolve 61 mg of CDNB in 5.0 ml of distilled alcohol (1mM CDNB)

Procedure

Table 5.2: Procedure for GST estimation

Reagents	**Blank(ml)**	**Test(ml)**
Buffer	1	1
DDW	1.65	1.55
GSH	0.3	0.3
Sample	-	0.1
CDNB	0.05	0.05

The absorbance is checked at 340nnm at intervals of 1 min for 5 min.

5.7 Estimation of LPO

It is measured by the method of Beuge and Aust (1978)

Principle

Free radicals can cause damage to Poly unsaturated fatty acids by Peroxidation mainly by OH^- and O_2^-. The product of Peroxidation is Malondialdehyde (MDA) which is identified to react with Thiobarbituric acid (TBA) to give pale red color absorbance at 535nm.

Reagents:

- 150mM Tris-HCl buffer(pH=7.1)
- 1.5mM Ascorbic acid
- 1mM $FeSO_4$
- 10% Trichloroacetic acid
- 0.375% Thiobarbituric acid pH=7)

Procedure

Table 5.3: Procedure of LPO estimation

Reagent	**Blank(ml)**	**Test(ml)**
Tris-HCl buffer	0.1	0.1
$FeSO_4$	0.1	0.1
Ascorbic acid	0.1	0.1
DDW	0.7	0.6
Sample	-	0.1
Incubated @ 37°C for 15 min and the reaction stopped by:		

TCA	**1**	**1**
TBA	2	2

Incubated for 15 min in boiling water bath followed by cooling and centrifuging at 500g for 10 min.

Took the supernatant and absorbance were taken at 532nm. LPO concentration was calculated and the result was expressed as n moles of MDA formed /ml.

5.8 Estimation of SOD

The SOD is estimated by the method of Kono (1978) in cell homogenate.

Principle

The principle lies in the reduction of Nitroblue Tetrazolium dye to formazon which is blue in color and governed by superoxide radicals. These radicals are produced by the auto-oxidation of hydroxylamine hydrochloride. In addition to SOD, it stops NBT from getting reduced and mediated by hydroxylamine hydrochloride. Enzyme activity is measured by the extent of inhibition.

Reagents

- 50mM Sodium Carbonate buffer in 0.1mM EDTA (pH=10.8)
- 96µM Nitro Blue Tetrazolium in 95% ethanol.
- 0.6% Triton X-100 in DDW
- 20mM Hydroxylamine HCl (pH=6.0)

Procedure

Table 5.4: Procedure of SOD estimation

Reagents	**Blank(ml)**	**Test(ml)**
Buffer	1.35	1.3
Hydroxylamine HCl	0.1	0.1
Triton X-100	0.1	0.1
NBT	0.5	0.5

Keep tubes for 2 min

Sample	**-**	**0.05**

The blue color that was developed was measured at 560nm at intervals of 30sec for 2min.

Calculations

The concentration of enzyme that gives a half-maximal inhibition of NBT reduction is known as one unit of the enzyme activity.

5.9 In vivo Animal studies

Rats were procured from NIPER, Mohali which were 7 weeks of age, and weighing approximately 160g (Protocol no: LPU/IAEC/2021/83). They were housed 6 and 7 per cage in a room with a 12/12-hour light/dark cycle and an ambient temperature of 22 to 25°C. After one week of adaptation, rats were haphazardly divided into two groups – control fed with a normal pellet diet (NPD) and HFD fed with HFD. After the duration of 16 weeks, the HFD rats were again divided into HFD, HFD +METFORMIN (positive control), HFD+UMB (low dose), HFD+UMB (high dose), HFD+GABA (High dose), HFD+GABA (low dose), Diet reversal, Diet reversal NCD+UMB (High dose), diet reversal NPD+UMB (low dose), diet reversal NPD+GABA (High dose), diet reversal NPD+GABA (low dose) and diet reversal metformin (Fig 5.3) (Table 5.5).

5.9.1 Development of diabetes

Table 5.5: Dosing in the animal groups

S.No	Group Name	Diet + Dose and route of drug treatment	No. of animals
Group 1	Vehicle control	NPD + 0.5% CMC (p.o.) (Vehicle)	6
Group 2	UMB *per se*	NPD + 40 mg/kg	6
Group 3	GABA oil *per se*	NPD + 200 mg/kg	6
Group 4	Negative control	HFD	7
Group 5	Positive control	HFD +100 mg/kg of metformin (p.o.)	7
Group 6	UMB low dose	HFD + 20 mg/kg (p.o.)	7
Group 7	UMB high dose	HFD + 40 mg/kg (p.o.)	7
Group 8	GABA low dose	HFD + 100 mg/kg (p.o.)	7
Group 9	GABA high dose	HFD + 200 mg/kg (p.o.)	7
Group 10	Diet reversal UMB low dose	NPD+ 20 mg/kg (p.o.)	7
Group 11	Diet reversal UMB high dose	NPD + 40 mg/kg (p.o.)	7
Group 12	Diet reversal GABA low dose	NPD+ 100 mg/kg (p.o.)	7
Group 13	Diet reversal GABA high dose	NPD+ 200 mg/kg (p.o.)	7
Group 14	Diet reversal Metformin	100 mg/kg of metformin (p.o.)	7

Group 15	Diet Reversal	HFD for 16 weeks and NPD for next 8 weeks	7
		TOTAL	102

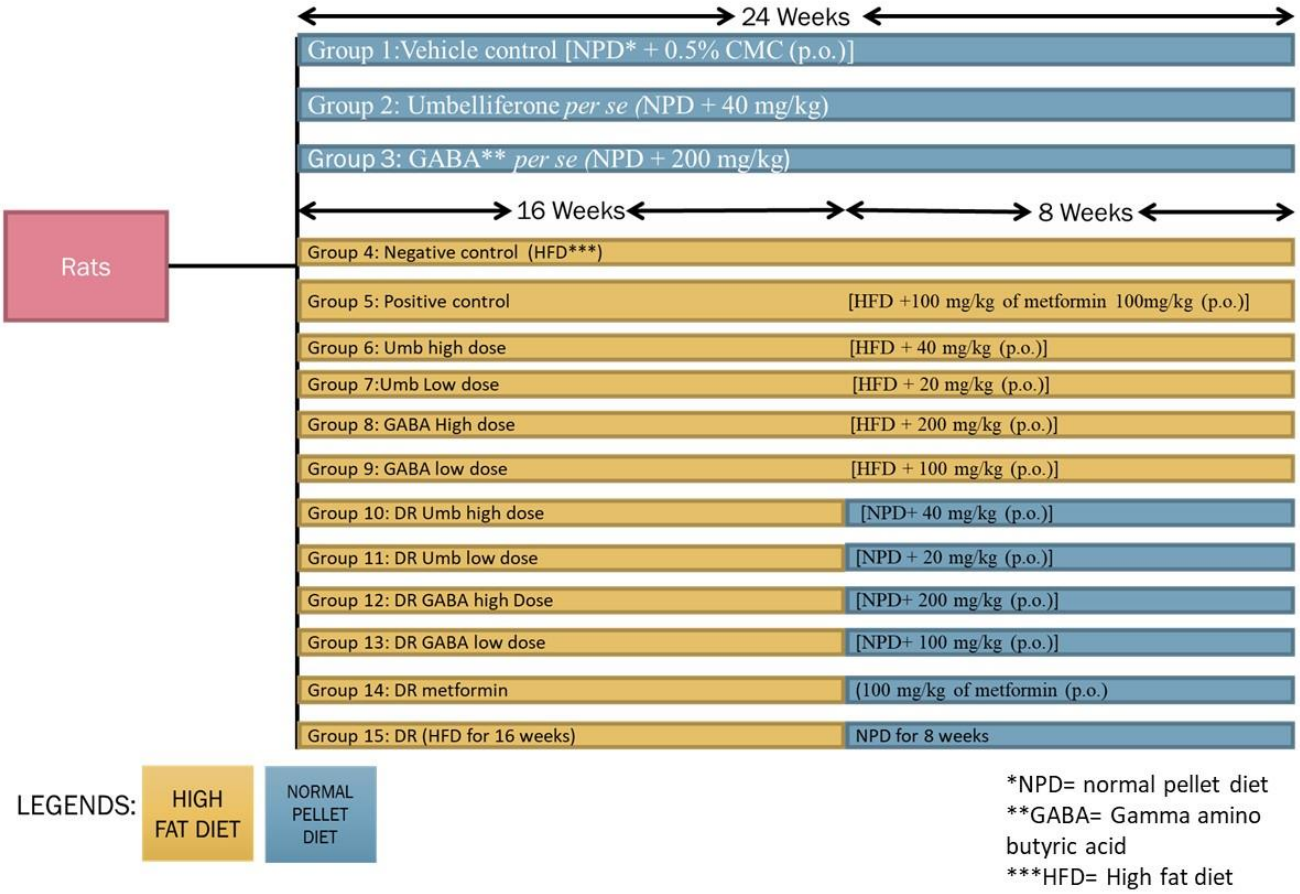


Figure 5.3: Timeline and dosing for animal study.

5.9.1.1 Parameter of evaluation

The parameters that were evaluated at the end of the studies to find the effect of the phytochemical treatment are as follows:

5.9.1.1.1 Bodyweight estimation

Before in vivo studies, the body weight estimation of the rats was carried out and after starting experimentation body weights were monitored every week before behavior assessment. The estimations were performed in triplicates.

5.9.1.1.2 Blood plasma glucose

The blood glucose estimation was done using the counter appliance accucheck by withdrawing 0.1ml of blood from the tail of mice.

5.9.1.1.3 Lipid profile test (LPT) and Renal profile test (RPT)

Lipid profiles include total cholesterol, HDL-cholesterol, triglyceride, and calculated LDL. Renal profiles include blood urea, serum creatinine, and serum uric acid. These assays were run in the clinical chemistry laboratory Gargi laboratory Jalandhar using a fully automated biochemistry and electrolyte analyzer (also for lithium) and ELISA system

Protocol

- Animals fasted for 12 hours and blood samples were collected in heparin-coated tubes.
- Blood samples were taken by the retro-orbital method. Plasma was separated from blood by centrifugation at 300 x g in a centrifuge.
- Plasma was stored at 4^{o}C and sent to Gargi laboratory Jalandhar for lipid, renal, and liver profile.

5.9.1.1.4 Biochemical parameters (Oxidative biomarkers)

Oxidative biomarkers: After the completion of the study on the last day of treatment, animals marked for biochemical studies were sacrificed by dislocating their cervical. The tissues removed and triturated to make a 10% (w/v) tissue homogenate in 0.1 M phosphate buffer (pH 7.4). The clear supernatant obtained after centrifugation at 400g (3000 rpm) for 15 minutes, was used for various biochemical studies to estimate the levels of CAT, GST, and LPO.

5.9.1.1.5 Histopathology

The kidneys were retrieved from SD rats from each group after sacrificing and immediately preserved in 10% formalin to avoid drying, at the end of the experiment Histopathological evaluation of various tissue sections was done with hematoxylin and eosin staining. The histopathological evaluation was performed in Gargi laboratory, Jalandhar.

5.10 SIRT1 ASSAY

5.10.1 PRINCIPLE OF THE ASSAY

The quantitative sandwich enzyme immunoassay method was used in this assay. An anti-SIRT1/SIR2L1 antibody had been pre-coated. Standards and samples are added to the wells, and the immobilized antibody binds to any SIRT1/SIR2L1 that may be present. A biotin-conjugated antibody specific for SIRT1/SIR2L1 was added to the wells after any unbound compounds have been removed. Avidin-conjugated Horseradish Peroxidase (HRP) was then added to the microplate after washing. A substrate solution was then added to the wells after a wash to discard any unbound avidin-enzyme reagent, and color develops in proportion to the amount of SIRT1/SIR2L1 bound in the initial phase. The growth of the color was halted, and the color's intensity is gauged.

5.10.2 Sample collection

One ml of 1X PBS was used to homogenize 100 mg of tissue before being refrigerated at -20°C overnight. The cell lysates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C after two freeze-thaw cycles were used to rupture the cell membranes. A supernatant was used to test.

5.10.3 Procedure

- In every well we added 100µl of standard and sample. Applied the provided sticky strip as a covering. At 37 °C, incubated for two hours.
- Removed the contents of each well without washing.
- Filled each well with 100µl of Biotin-antibody (1x) and incubated at 37 °C, for one hour.

- Removed all contents and washed for a total of three washes. For washing, filled each well with 200µl of Wash Buffer using a squirt bottle and let it stand for 2 minutes. Removed any leftover wash buffer after the last wash by aspirating or decanting.
- Filled each well with 100µl of HRP-avidin (1x), followed by incubation at 37 °C, for one hour.
- Washed again for 5 times.
- To each well, we added 90µl of TMB substrate and incubated at 37 °C for 15–30 minutes by shielding against the light.
- Then added 50µl of Stop Solution.
- Using a microplate reader with the wavelength set to 450 nm, determined each well's optical density in 5 minutes.

5.10.4 Statistical analysis

All the results were persuasive as mean ± SEM. The one-way analysis of variance (ANOVA) followed by the Tukey test using Sigma stat software was used to analyze the statistics of behavioral and biochemical data.The difference was considered to be significant when the level of significance was 5% ($p<0.05$).

5.11 Histone extraction

5.11.1 Reagents

Low salt buffer (pH 7.4)

Tris 10mM, NaCl 10mM, EDTA 10mM, 1mM Sodium orthovanadate, NaB and phenylmethylsulfonyl fluoride (PMSF)

Extraction buffer

0.2 M HCL and 20% TCA

Acetone

5.11.2 Histone isolation protocol

In low salt buffer (LSB), the nuclear pellet was suspended and 22.7 µl of 0.2 M HCl was added to it which was then subjected to sonication at 40-50 kHz for 10 seconds at an interval of 1 minute and repeat sonication thrice. Then centrifuge the mixture for 30 min at 11000g (13000rpm) and keep the supernatant. To the supernatant add 20%TCA and vortex to ensure proper mix-up and then keep in the ice bath for 30 min. The mixture was again centrifuged again for 30 min at 11000g (13000 rpm). Suspended pellet in 1 ml of 0.25% acetone-HCl, followed by vortexing and centrifuging again at 11000g (13000 rpm) for 30 min and repeated again. The tubes were covered with parafilm and kept at 0°C overnight. Pellet was then suspended in chilled distilled water.

5.11.3 Western blotting protocol

Principle

It utilizes the simple principle in which first the protein is separated according to molecular weight through electrophoresis and then followed by binding of this protein to the support called blot which allows its detection with the help of antibodies specific to the protein. It was done using protocol established by Tikoo et al., 2007a,b

Reagents

Separating gel buffer, pH 8.8(100ml)

- Tris-18.2 gm Set pH 8.8
- Sodium dodecyl sulfate (SDS) -400mg

Stacking gel buffer, pH 6.8(100ml)

- Tris-6.05gm Set pH 6.8
- SDS -400mg

Acrylamide 30x(100ml)

- Acrylamide -29.2gm
- Bis-acrylamide -0.8gm

Staining buffer (500ml)

- Methanol-250ml
- Acetic acid-50ml
- Brilliant blue-1.5g

Destaining buffer(500ml)

- Methanol-250ml
- Acetic acid-50ml
- Water -250ml

4x Sample buffer(10ml)

- 1M Tris -0.2g
- SDS -0.8g
- Glycerol -4ml
- B-mercaptoethanol -4ml
- 10% Bromophenol blue -10µl
- APS -1ml (100mg in ml water)

1x Running buffer(1 litre)

- Tris 3 gm
- Glycine 14.4 gm
- SDS 1 gm

Table 5.6: Components and their amount in the preparation of stacking gel and separating gel

Reagents	Stacking Gel	Separating gel
Water	2.83 ml	6.10 ml
Separating buffer	2.5 ml	2.5 ml
30% acrylamide	4.7 ml	1.3 ml
APS	33.3 µl	50 µl
TEMED	6.7 µl	10 µl
	Polymerise for half an hour	

5.11.4 Protocol

First step:

SDS-PAGE

Extracted 0.5 µg histone sample was taken and suspended in a sample buffer. Samples were heated at 95ºC for 5 minutes, Samples were centrifuged to restore the sample volume. Stalking buffer and separating buffer were prepared. Histone samples were loaded in the wells per lane, also pre-stained protein standard was loaded. The gel was run at a voltage of 70 V till stalking and after that gel was run at 140 V for 1-2 hours

Setup for transfer

- A transfer buffer was prepared in order to fill the tank while the transfer process
- After successful SDS-PAGE, the gel was removed from the cassette. The stalking and well area of gel was cut off.
- The gel was put in a transfer buffer, and shaken for 10 minutes.
- The nitrocellulose membrane was soaked in the transfer buffer for 30 minutes.**Second step-**

Western blotting

Reagents

Transfer buffer 500ml

- Tris -1.5gm
- Glycine -7.2 gm
- Distilled water (make up volume 500ml)

Tris-Buffered saline (**TBS)** (pH 7.2)

- 5M Nacl-30ml
- 2M tris-10ml
- Milli que water -955 ml

TBST (pH 7.2)

- TBS-500ml
- Added -0.5% Tween 20

2X Blocking buffer

- BSA(Bovine serum albumin)-400mg in 20ml TBS (for 1 blot)

 TBS-500ml

Primary antibody

Secondary antibody

5.11.5 Transfer assembly

 The cassette was taken and opened to make an assembly

 The sandwich was made by placing a foam pad on one side of the cassette followed by a sheet of filter paper followed by gel on top of the filter paper.

 On top of the gel, the nitrocellulose membrane was applied, followed by the second sheet of filter paper, then the second pad of foam pad.

 The cassette was closed after making this sandwich.

5.11.6 Transfer method

 The cassette that has a sandwich assembly was placed in the transfer tank.

 The cathode (-) should face the gel side of the cassette, while the membrane side should face the anode.

 A transfer buffer was added to the transfer tank up to the mark.

 The apparatus was run at 10-15 V for 1 to 2 hours till the transfer was complete.

 After transfer, the sandwich was removed from the cassette and carefully disassembled using forceps.

 After that ponceau staining was used to verify the successful transfer of histone proteins

5.11.7 Processing of western blot.

 Blocking – In this step, we used BSA as a blocking buffer. The membrane was blocked by placing it in a blocking buffer for 2 hrs at room temperature (RT) using 2X BSA/0.1% tris buffer saline (TBS) while continuing shaking.

 Incubation with primary antibody- The membrane was incubated at 4ºC overnight with the antibody.

 Washing- The blot was washed with 0.1% of tris buffer saline tween TBST 4 times. Washing was done 2 times for 5 minutes followed by 2 times 10 min washes.

 Incubation with secondary antibody- The membrane was incubated with a secondary antibody for 1hr with continuous shaking @ 37ºC.

 Washing - The blot was washed with 0.1% of tris buffer saline tween TBST 4 times. Washing was done 2 times for 5 minutes followed by 2 times 10 min washes.

 The blot was developed.

5.11.8 Detection

The ECL (enhanced chemiluminescence) technology and ECL Hyperfilm were used to detect proteins (Amersham Biosciences). Quantification of western blots was done by densitometric analysis and the exposures have a linear dynamic range. The Image J software was used for densitometric analysis.

5.11.9 Histone western blotting antibodies

Analysis of western blot was carried out employing the following antibody:

 Rabbit anti- [aceylated histone H3 (Lys9/14)] antibody (1:5000 dilution; Santa Cruz Biotechnology).

- Rabbit anti-histone H3 antibody (1:5000 dilution; Santa Cruz Biotechnology).
- and HRP-conjugated anti-rabbit secondary antibodies (diluted 1:20000; Santa Cruz Biotechnology).
- Anti-SIRT1 antibody. (Santa Cruz Biotechnology).

Chapter 6

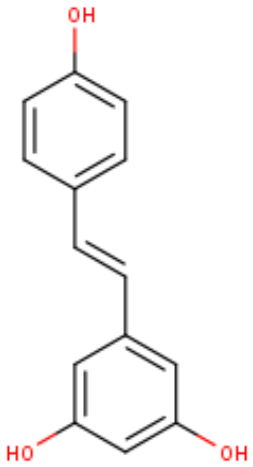
Results

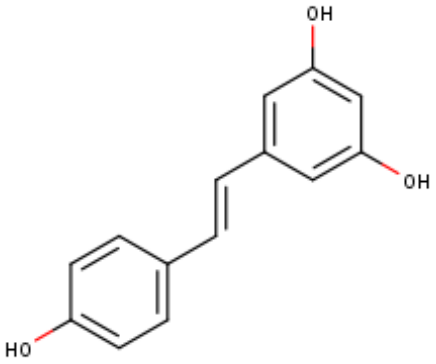
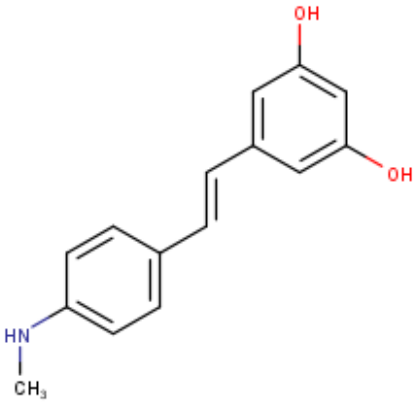
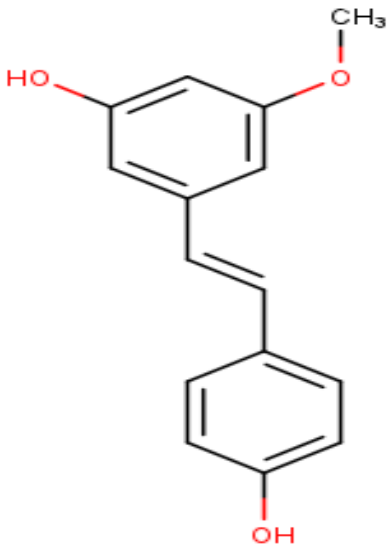
6.1 Data mining and similarity searching

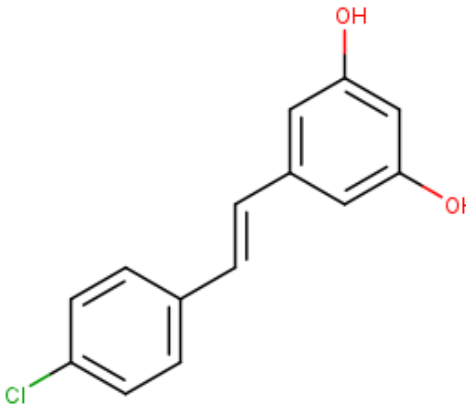
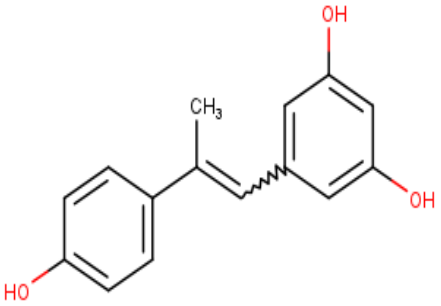
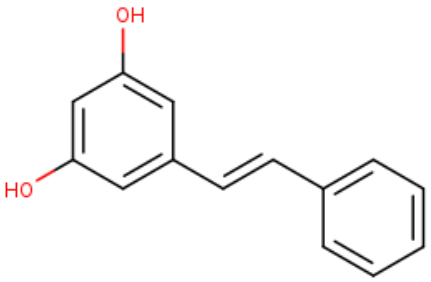
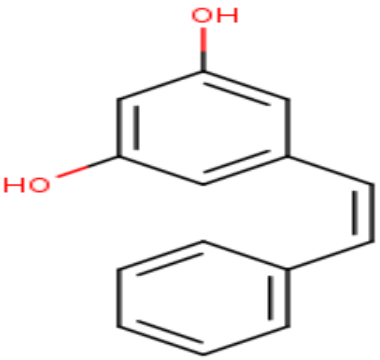
According to scientific evidence, if two molecules are structurally similar, they may share biological functions (Martin et al., 2002). To fish out novel phytochemicals that can reverse DN and GM following the hypothesis that "similar structures have similar biological activities" we found structurally similar molecules with already reported phytochemicals that can reverse DN and MM. For this, we started with data mining succeeded by structural similarity searching and Autodock which was concluded by Swiss ADME studies. The reported phytochemical's structure was taken as a query molecule to search databases (using PubChem fingerprint Tanimoto-based 2-dimensional similarity search (70%) Tanimoto ~0.7 as a filter) to get structurally similar compounds.

Based on the literature review we chose 6 natural epigenetic modifiers **Metformin, Resveratrol, Vanillic Acid, Esculetin, Genistein, and Berberine,** and searched for similar compounds. We got several similar compounds; the list is attached below in a table.

Table 6.1: Compounds similar to Resveratrol

Similar compound	Similarity %	ChEMBL ID	Structure
Resveratrol		ChEMBL165	

5-(1-(4-Hydroxyphenyl)prop-1-en-2-yl)benzene-1,3-diol	80.59	CHEMBL491080	
5-[(E)-2-[4-(Methylamino)phenyl]ethenyl]benzene-1,3-diol	80.95	CHEMBL2208044	
3-methoxyresveratrol	82.03	CHEMBL498917	

5-[(1E)-2-(4-Chlorophenyl)ethenyl]-1,3-Benzenediol	82.09	CHEMBL3827411	
5-(2-(4-Hydroxyphenyl)prop-1-enyl)benzene-1,3-diol	83.03	CHEMBL474127	
Pinosylvin	83.58	CHEMBL101506	
Cis-Pinosylvin	83.58	CHEMBL2203685	

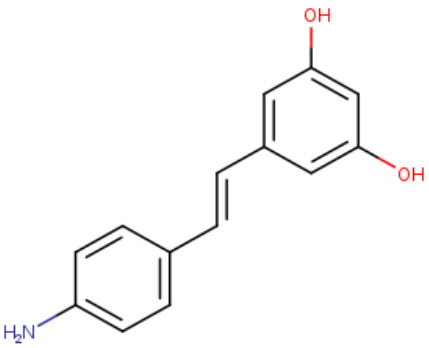
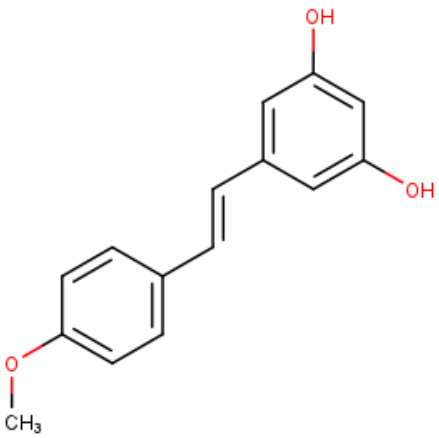
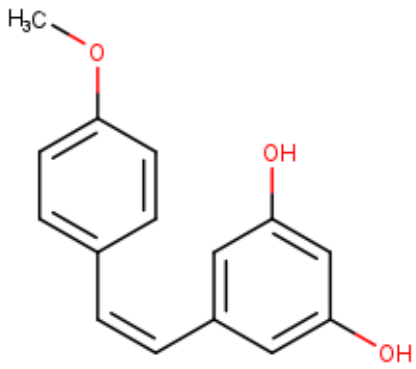
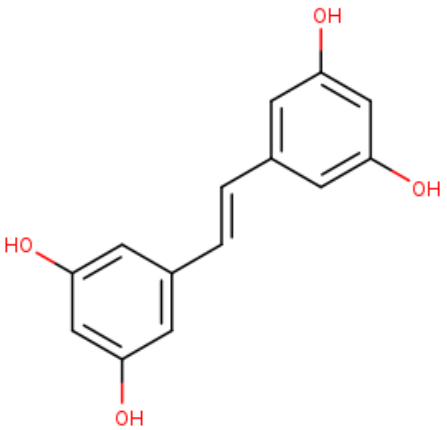
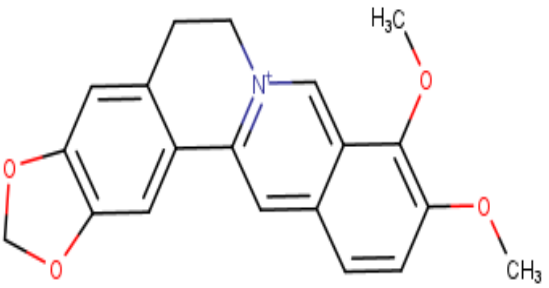
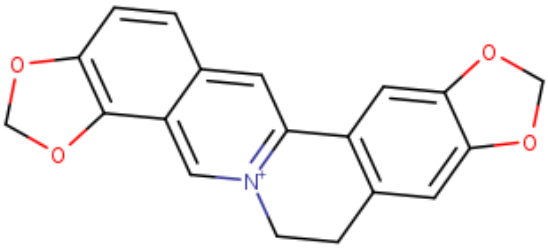
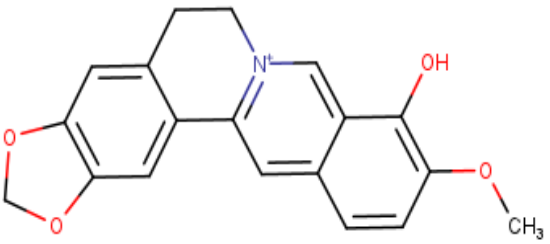
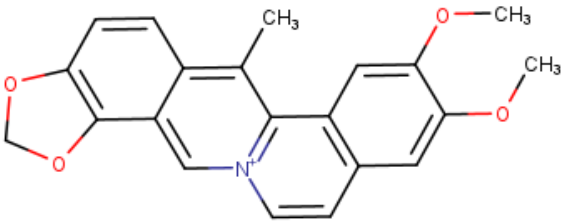
5-[(E)-2-(4-Aminophenyl)ethenyl]benzene-1,3-diol	84.72	CHEMBL1172402	
RESVERATROL 4'-METHYL ETHER	87.29	CHEMBL291501	
5-[(Z)-2-(4-Methoxyphenyl)ethenyl]benzene-1,3-diol	87.29	CHEMBL3136887	
5-[2-(3,5-Dihydroxyphenyl)-(E)-1-ethenyl]-1,3-benzenediol	87.54	CHEMBL1016559	

Table 6.2: Compounds similar to Berberine:

Similar compound	Similarity %	ChEMBL ID	Structure
Berberine		CHEMBL295124	
COPTISINE	93.76	CHEMBL362071	
BERBERRUBINE	97.83	CHEMBL203135	
DEHYDROCAVIDINE	96.47	CHEMBL1187569	

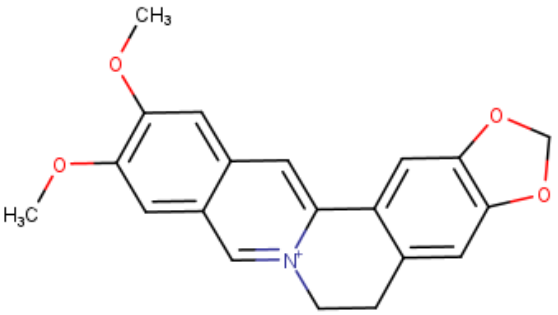
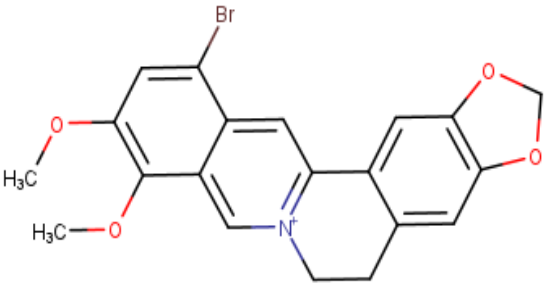
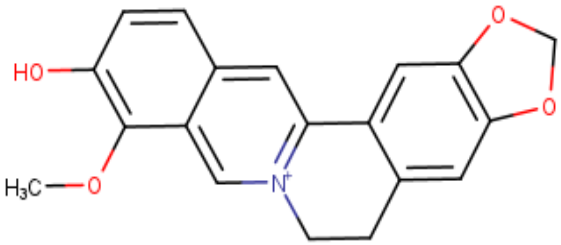
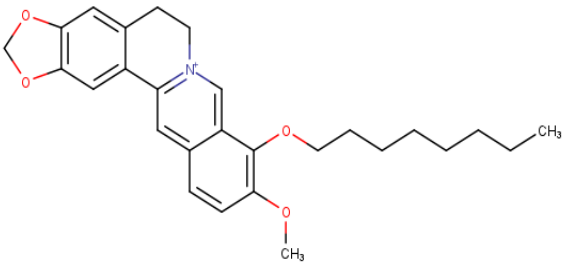
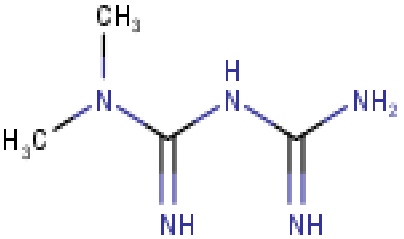
PSEUDOBERBERINE	97.36	CHEMBL1186186	
12-Bromoberberine Chloride	95.61	CHEMBL1186321	
THALIDENDINE	98.01	CHEMBL1187148	
9-O-OCTYL-BERBERINE	93.24	CHEMBL2365815	

Table 6.3: Similar compounds of Metformin

Similar compound	Similarity %	ChEMBL ID	Structure
Metformin		ChEMBL1431	

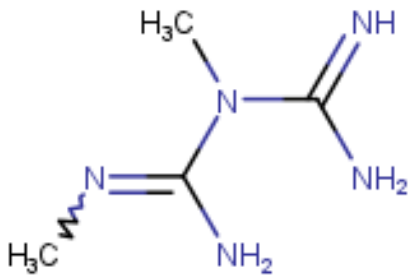
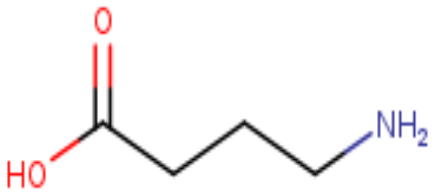
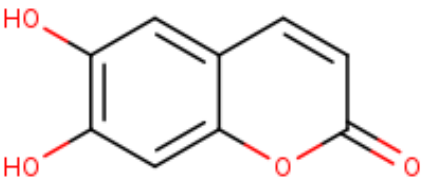
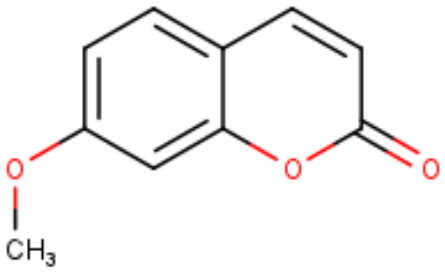
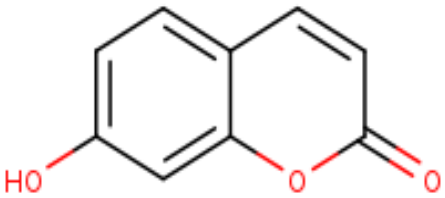
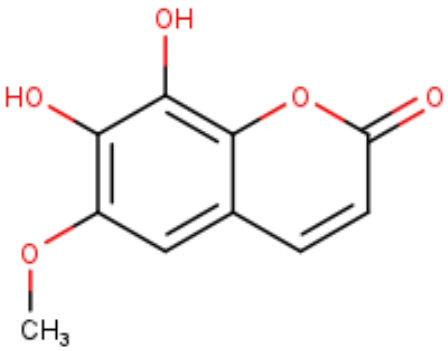
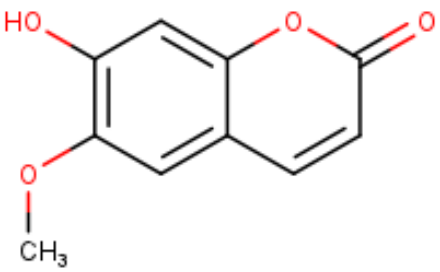
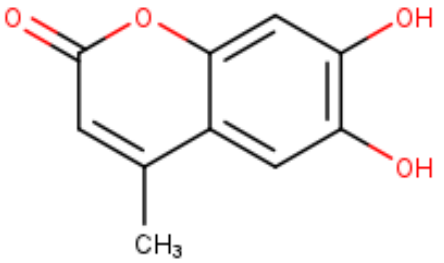
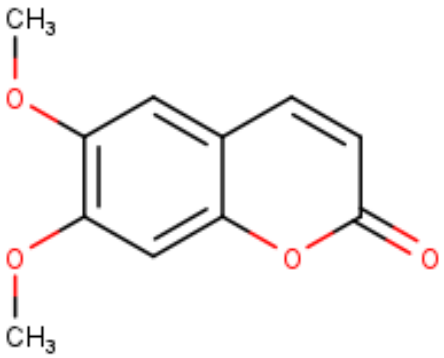
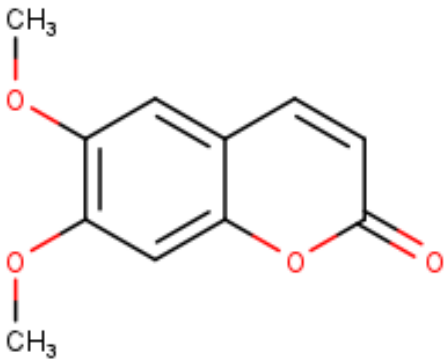
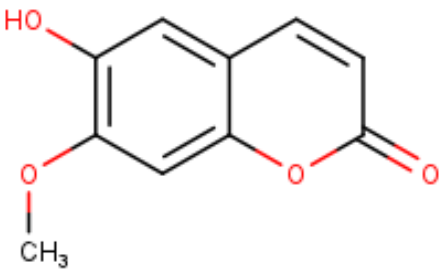
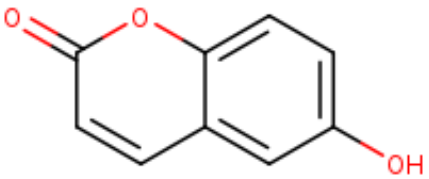
1-Carbamimidoyl-1,2-dimethylguanidine	**86.53**	ChEMBL1187231	
4-aminobutanoic acid	**82.50**	ChEMBL2348412	

Table 6.4: Similar compounds to Esculetin:

Similar compound	Similarity %	ChEMBL ID	Structure
Esculetin			
HERNIARIN	80.77	CHEMBL49732	
UMBELLIFERONE	84.3	CHEMBL51628	
FRAXETIN	85.16	CHEMBL54909	
SCOPOLETIN	94.48	CHEMBL71851	

4-METHYLESCULETIN	87.27	CHEMBL313244	
SCOPARONE	90.18	CHEMBL325864	
ETHOXYCOUMARIN	80	CHEMBL191528	
ISOSCOPOLETIN	94.12	CHEMBL390320	
6-HYDROXYCOUMARIN	80.47	CHEMBL243263	

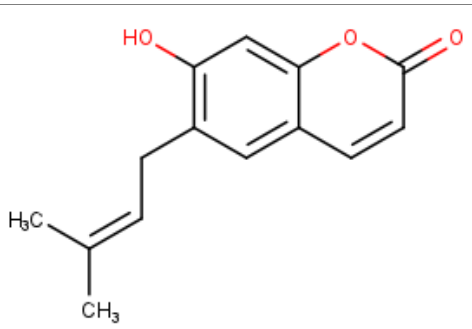
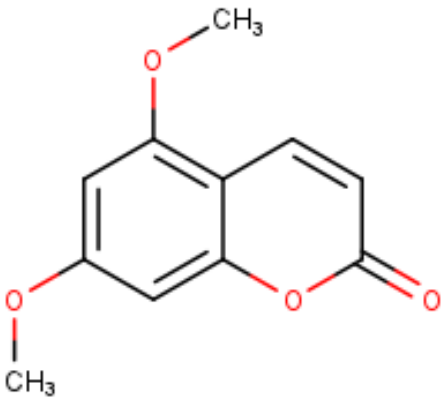
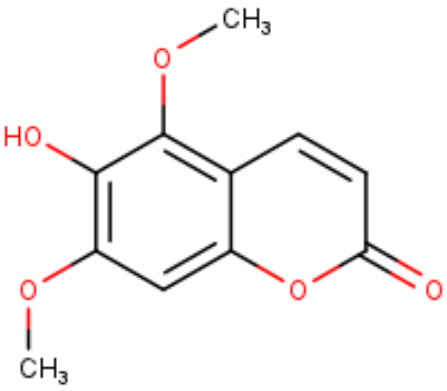
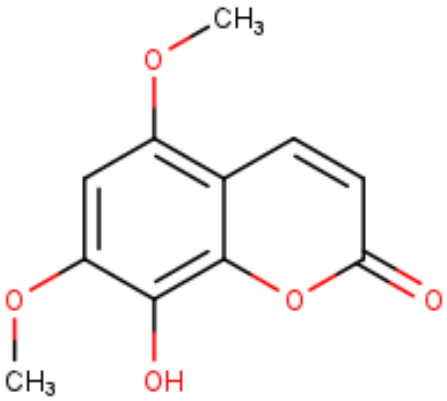
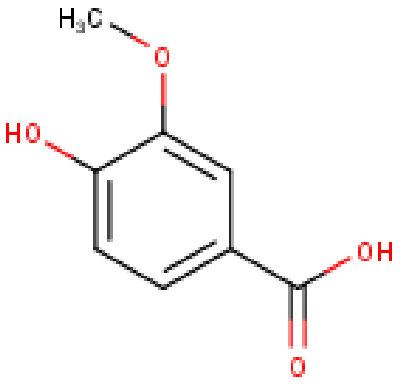
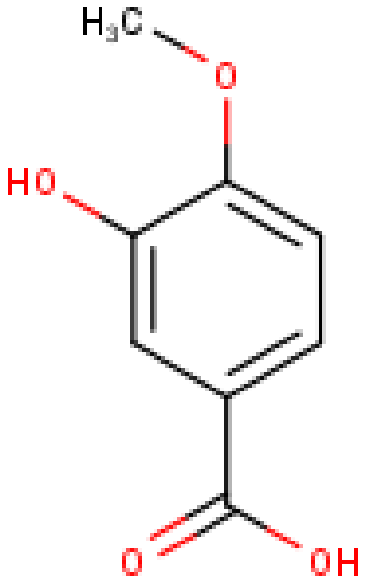
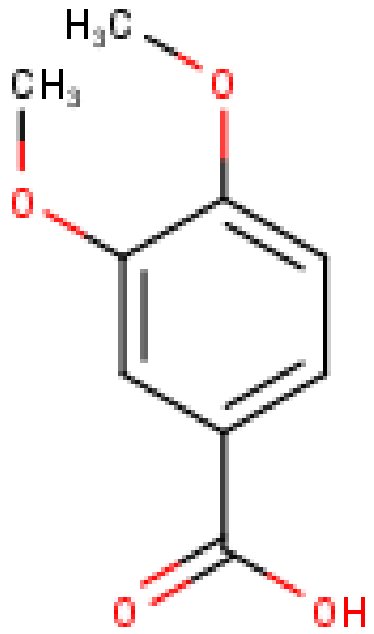
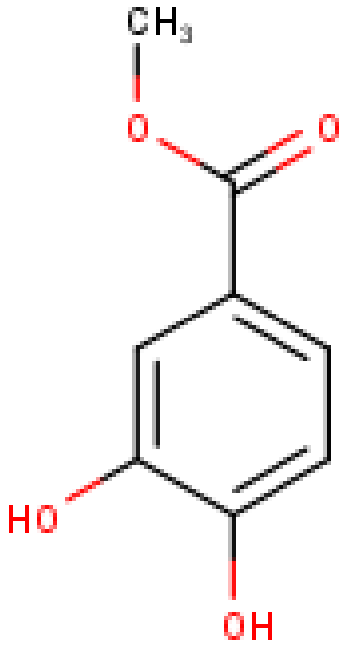
DEMETHYLSUBEROSIN	81.45	CHEMBL502689	
CITROPTEN	82.56	CHEMBL481049	
FRAXINOL	82.91	CHEMBL611349	
LEPTODACTYLONE	80.33	CHEMBL595334	

Table 6.5: Similar compounds to Vanillic Acid

Similar compound	Similarity %	ChEMBL ID	Structure
Vanillic Acid		**ChEMBL1120658**	
ISOVANILLIC ACID	97.86	CHEMBL88700	

VERATRIC ACID	84.49	CHEMBL118903	
METHYL PROTOCATECHUATE	84.05	CHEMBL486027	

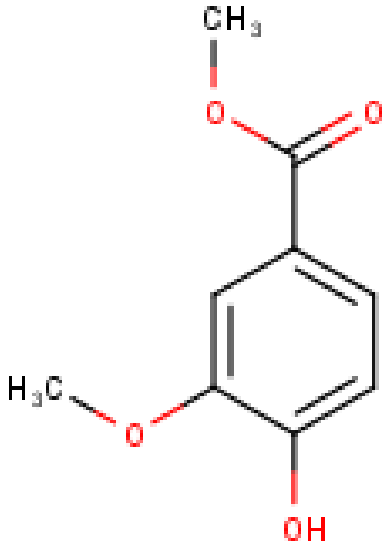
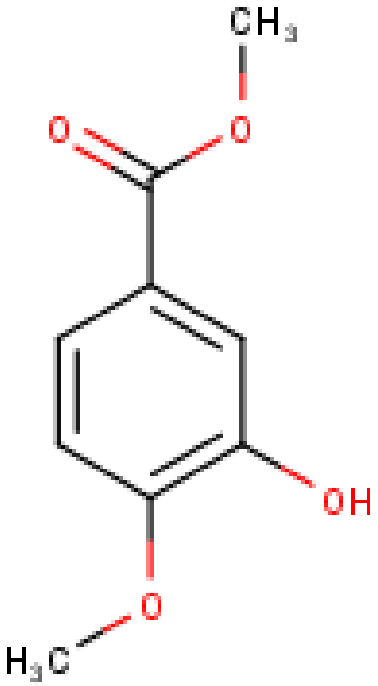
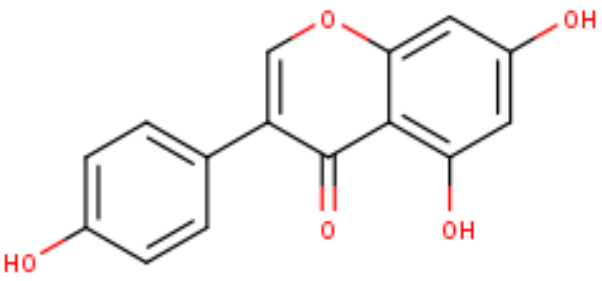
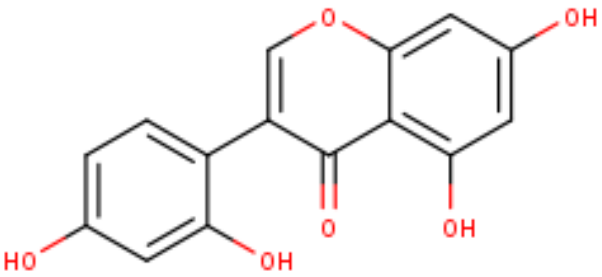
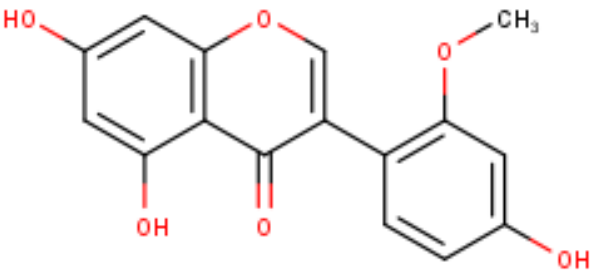
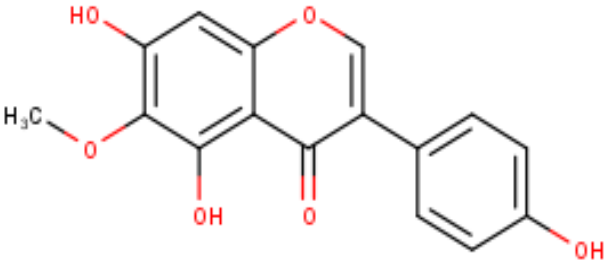
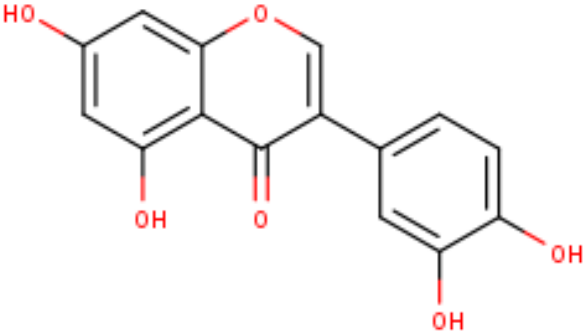
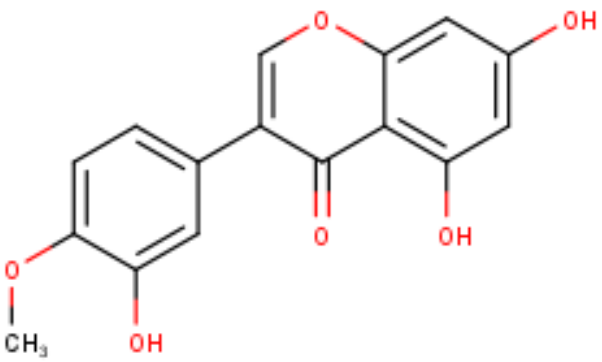
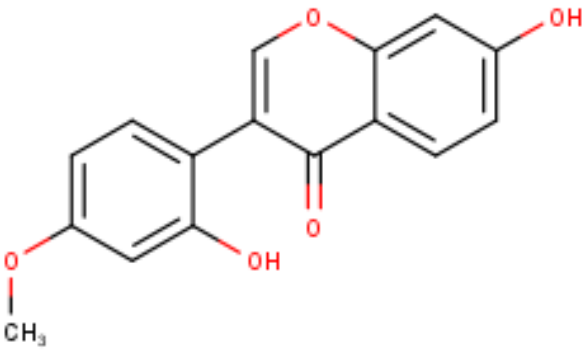
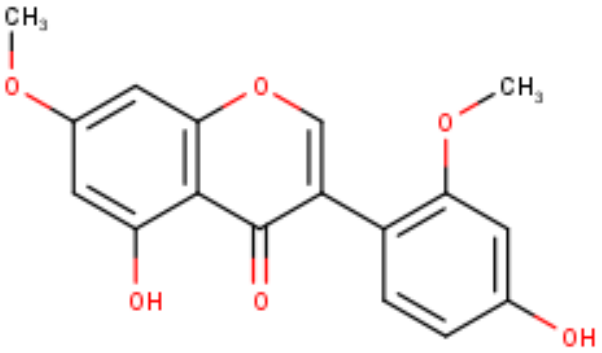
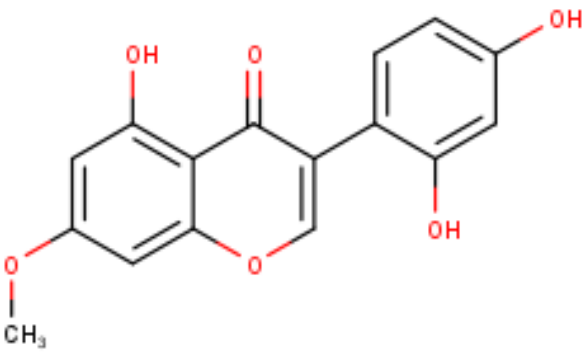
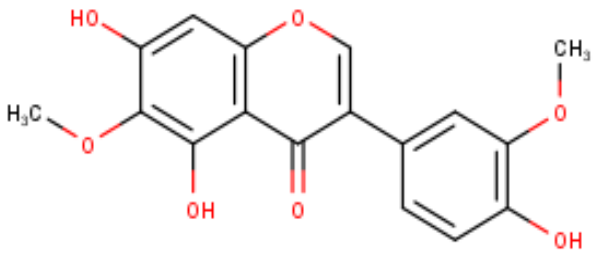
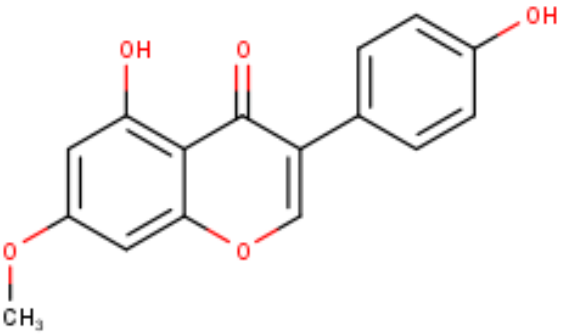
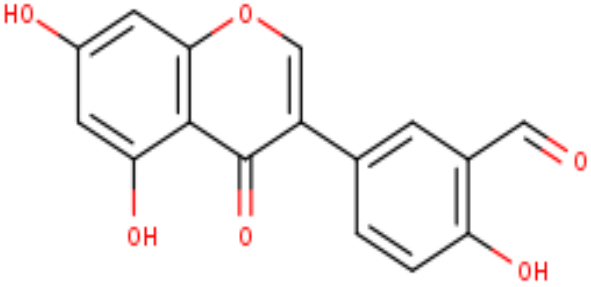
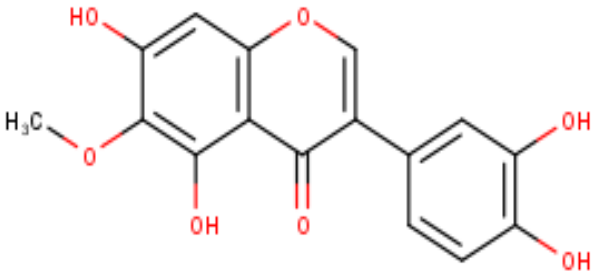
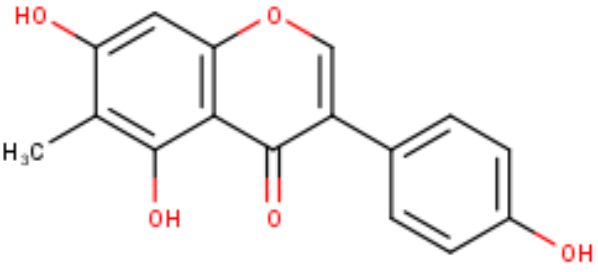
METHYL VANILLATE	82.57	CHEMBL486214	
ISOVANILLIC ACID METHYL ESTER	80.72	CHEMBL2252125	

Table 6.6: Similar compounds to Genistein

Similar compound	Similarity %	ChEMBL ID	Structure

Genistein		**CHEMBL44**	
2'-Hydroxygenistein	94.52	CHEMBL6665	
THERALIN	93.31	CHEMBL7348	
TECTORIGENIN	93.12	CHEMBL242740	
OROBOL	93.22	CHEMBL241609	

PRATENSEIN	92.13	CHEMBL252904	
XENOGNOSIN B	90.03	CHEMBL253514	
ERIOPHORIN C	90.71	CHEMBL469830	
CAJANIN	92.85	CHEMBL469630	

IRISTECTORIGENIN A	90.11	CHEMBL4868225	
PRUNETIN	96.07	CHEMBL4911174	
3'-formylgenistein	94.14	CHEMBL4518756	
IRILIN D	90.2	CHEMBL4699927	
6-Methylgenistein	93.68	CHEMBL5647669	

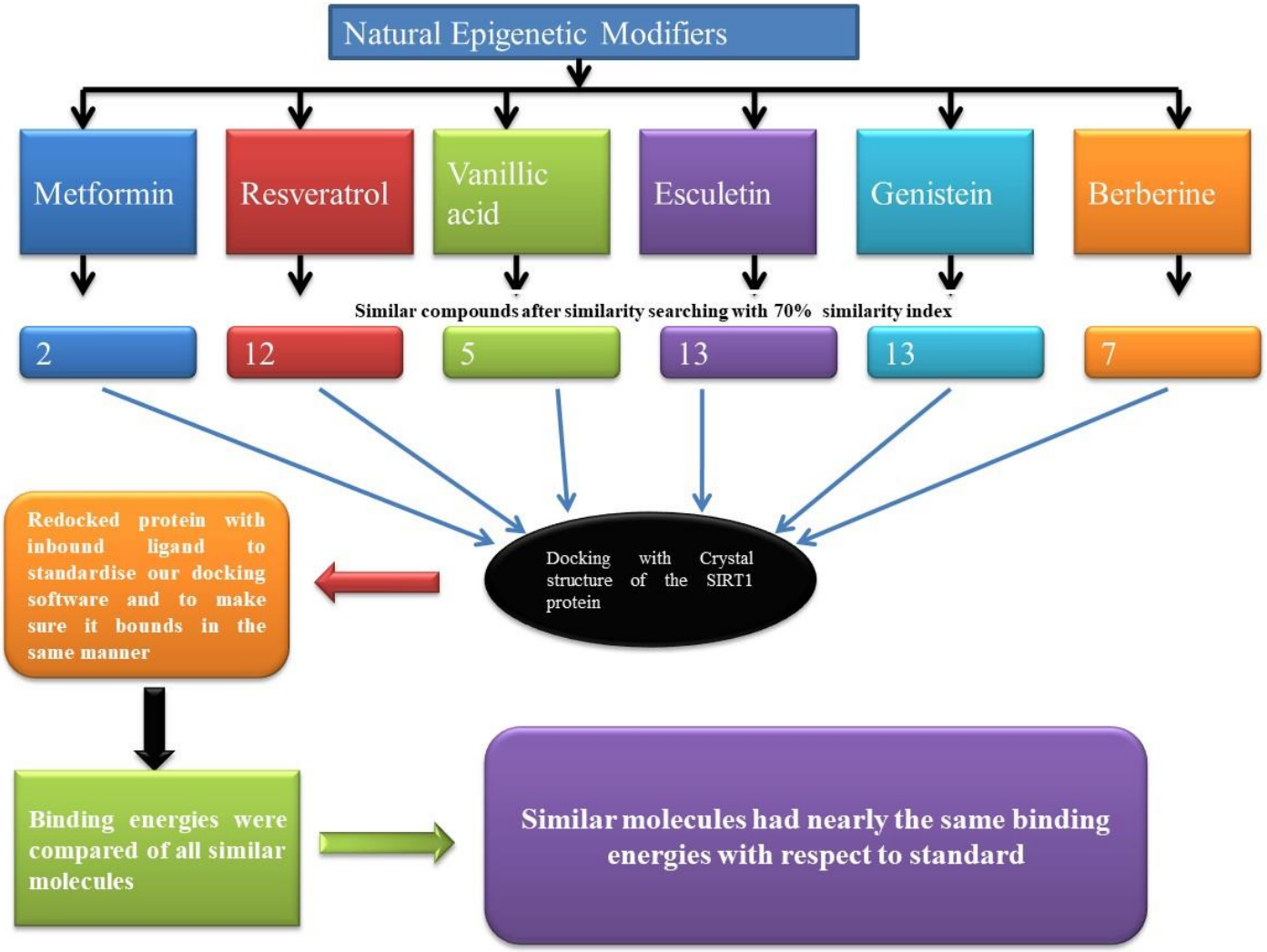


Figure 6.1: Phytochemicals selected for further in silico evaluation

6.2 Molecular docking

Following a thorough assessment of the literature, we selected phytochemicals that were promising candidates. Prediction of the query structure's binding conformations at the **Crystal structure of the SIRT1 catalytic domain bound to NAD and an EX527 analogue** (**SIRT1 protein PDB ID:4I5I)** active site was done using a technique called molecular docking (Grosdidier et al., 2011). (Figure 6.2).

The binding energy when resveratrol bound with the protein was -10.3kcal/mol and has amino acids PHE413, PHE297, PHE273, ILE411, and ILE 347 (Figure 6.3) participating in the binding and energy obtained for all other binding modes can be seen in Table 6.8. Phytochemical 5-(1-(4-Hydroxyphenyl) prop-1-en-2-yl)benzene-1,3-diol had the highest binding energy of -12.2kcal/mol (Table 6.9) and amino acids involved in the binding are ILE316, PHE273, PHE297, PHE413, ILE411, and VAL412 (Figure 6.4).

Binding energy when metformin docked with the protein was found to be -5.7kcal/mol, PHE273, GLN345, ILE347, HIS363 and VAL412 (Figure 6.5) are the surrounding amino acids. Other binding modes of metformin

are shown in Table 6.9 while Table 6.10 enlists the binding energy of similar compounds of metformin, where 1-Carbamimidoyl-1,2-dimethylguanidine had the highest binding energy of -5.8kcal/mol (Figure 6.6).

When protein was docked with berberine it had binding energy of -12.3kcal/mol with ILE411, ILE 347, PHE413, ILE279, ILE270, PHE273, and ILE316 (Figure 6.7) amino acids involved in the binding while other binding modes are shown in Table 6.11. The binding energy of similar compounds is shown in Table 6.12 where Berberrubine had the highest energy of -15.4kcal/mol (Figure 6.8).

When esculetin was docked with the protein we got -9.2kcal/mol of binding energy and all other binding modes are enlisted in Table 6.13. Amino acids involved in the binding of Esculetin and protein are ILE411, PHE413, VAL412, PHE297, PHE273, and ILE347 (Figure 6.9) Citropten had the highest binding energy of -11.3kcal/mol (Figure 6.10) and binding energy of other similar compound are shown in Table 6.14.

Vanillic acid had a binding energy of -11.9kcal/mol and the binding energy for all modes is given in Table 6.15. Amino acids involved in the binding of Vanillic acid with protein are ILE347, ILE411, GLN345, ASN346, PHE297, and PHE273 (Figure 6.11). Isovanillic Acid Methyl Ester had the highest binding energy of -7.6kcal/mol (Figure 6.12) among all other similar compounds (Table 6.16).

Genistein had a binding energy of -11.9kcal/mol and the binding energy for all modes is given in Table 6.17. Amino acids involved in the binding of Vanillic acid with protein are ILE411, VAL412, PHE297, and PHE273 (Figure 6.12). 3'-formylgenistein had the highest binding energy of -12.9kcal/mol (Figure 6.13) among all other similar compounds (Table 6.18).

Table 6.7: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with the standard ligand.

Binding modes	Affinity (kcal/mol)
1	-11.8

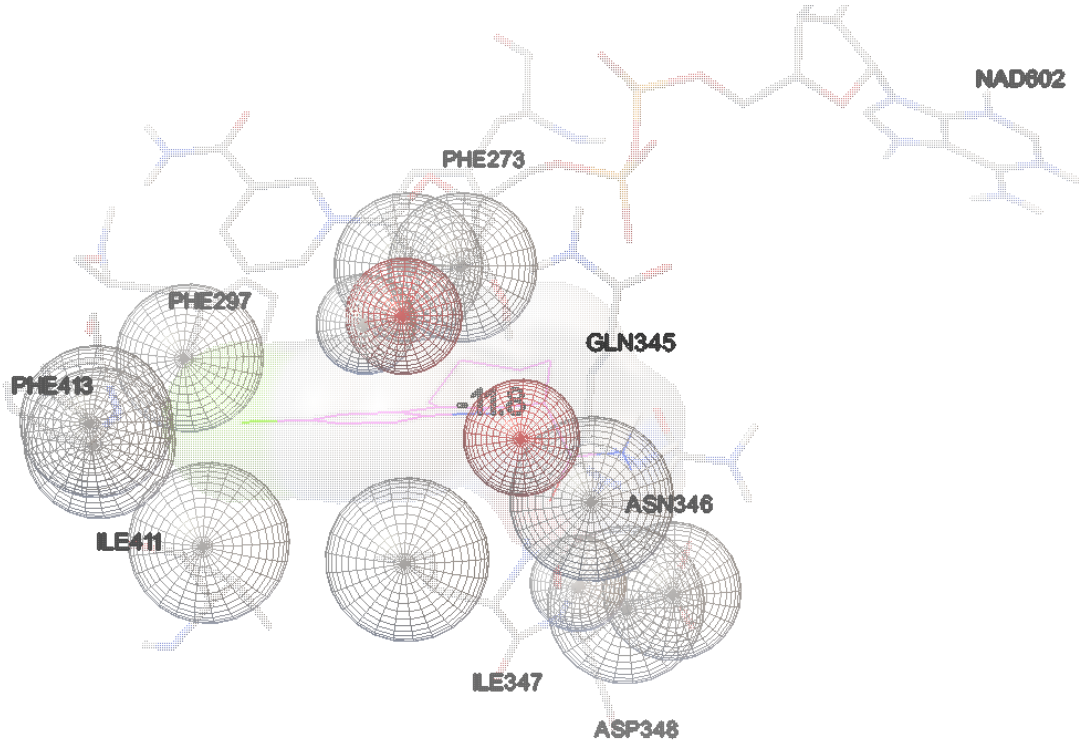


Figure 6.2: Docking of the SIRT1 protein (PDB ID:4I5I) with the standard ligand.

Table 6.8: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with resveratrol

Binding modes	Affinity (kcal/mol)
1	-10.3
2	-10.0
3	-9.4
4	-8.8
5	-7.8
6	-7.8
7	-7.7
8	-7.6
9	-7.5

Table 6.9: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with compounds similar to RESVERATROL

Compound Name	Binding energy (kcal/mol)
5-(1-(4-Hydroxyphenyl)prop-1-en-2-yl)benzene-1,3-diol	-12.2

RESVERATROL 4'-METHYL ETHER	-11.6
(E)-3,3',5'-Trihydroxystilbene | Trans-3,3',5-Trihydroxystilbene	-11.6
3-methoxyresveratrol	-11.6
5-[(1E)-2-(4-Chlorophenyl)ethenyl]-1,3-Benzenediol	-11.6
5-[(Z)-2-(4-Methoxyphenyl)ethenyl]benzene-1,3-diol	-11.5
5-[(E)-2-(4-Aminophenyl)ethenyl]benzene-1,3-diol	-11.5
Cis-Pinosylvin	-11.5
Pinosylvin	-11.4
5-[2-(3,5-Dihydroxyphenyl)-(E)-1-ethenyl]-1,3-benzenediol	-10.4
5-[(E)-2-[4-(Methylamino)phenyl]ethenyl]benzene-1,3-diol	-9.4
5-(2-(4-Hydroxyphenyl)prop-1-enyl)benzene-1,3-diol	-8.3

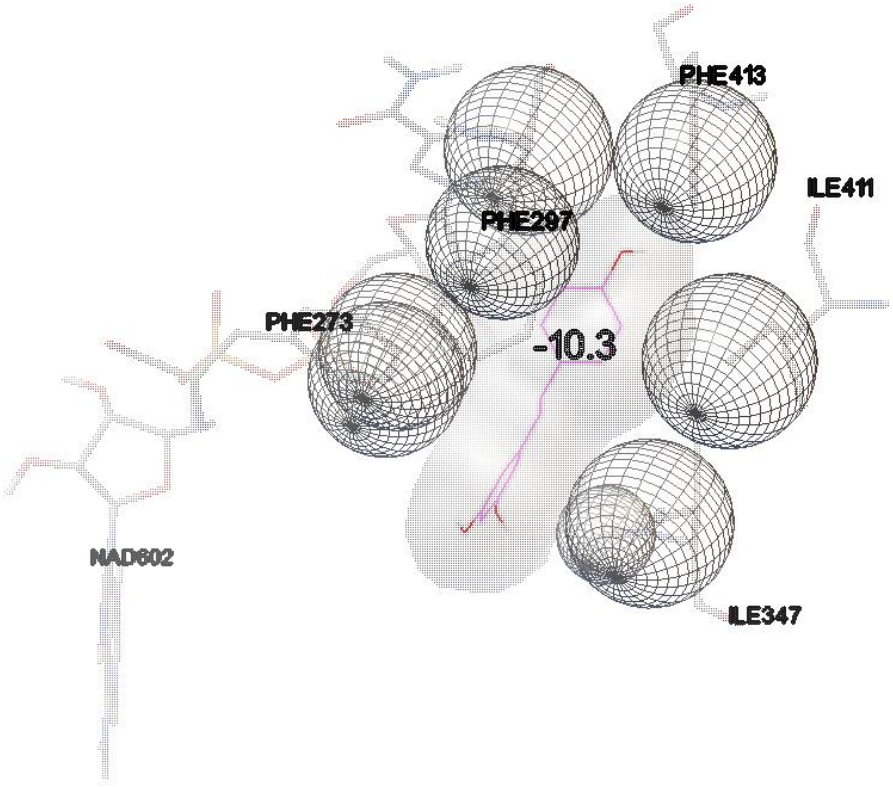


Figure 6.3: Docking of the SIRT1 protein (PDB ID:4I5I) with resveratrol

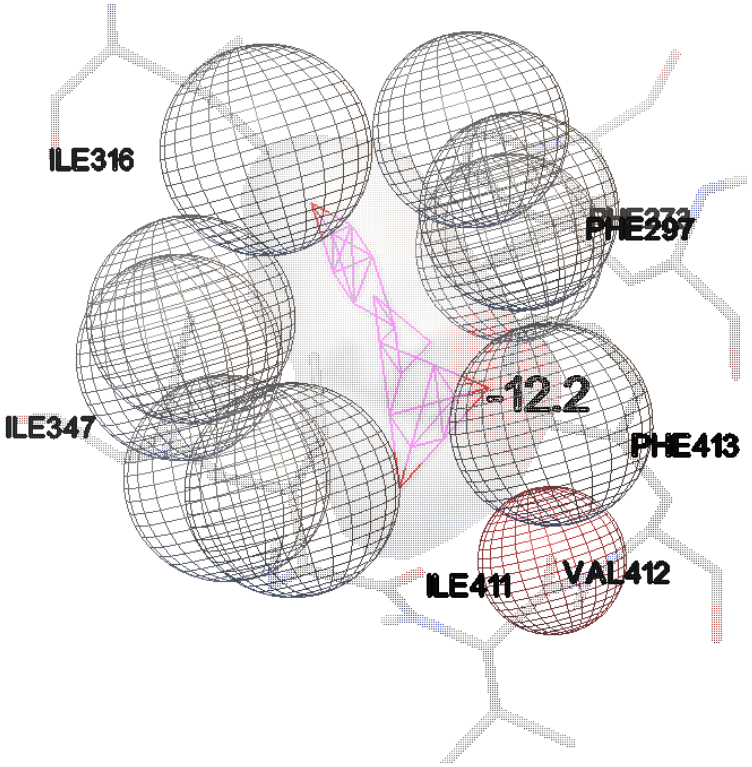


Figure 6.4: Docking of the SIRT1 protein (PDB ID:4I5I) with 5-[2-(3,5-Dihydroxyphenyl)-(E)-1-ethenyl]-1,3-benzenediol (Similar compound of Resveratrol, having highest binding energy)

Table 6.10: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with Metformin and with compounds similar to Metformin

Binding modes	Affinity (kcal/mol)	Compound Name	Binding energy (kcal/mol)
1	-5.7	1-Carbamimidoyl-1,2-dimethylguanidine	-5.8
2	-5.2	4-aminobutanoic acid	-4.5

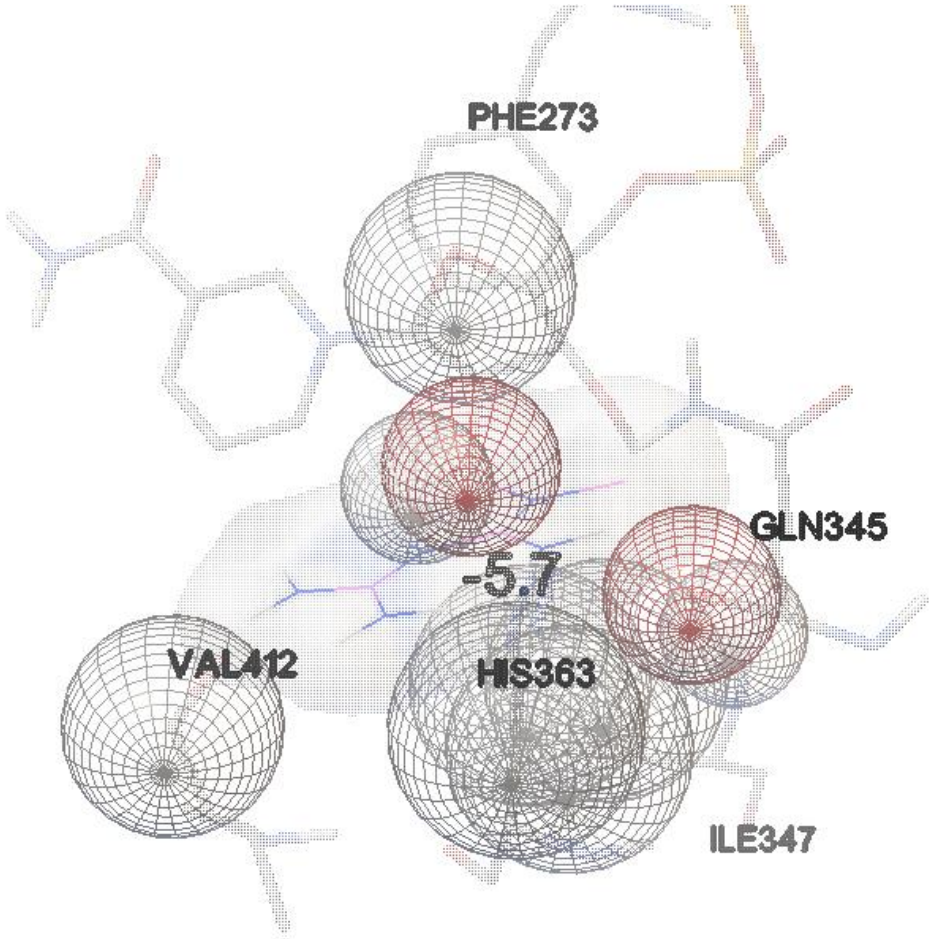


Fig 6.5: Docking of the SIRT1 protein (PDB ID:4I5I) with metformin

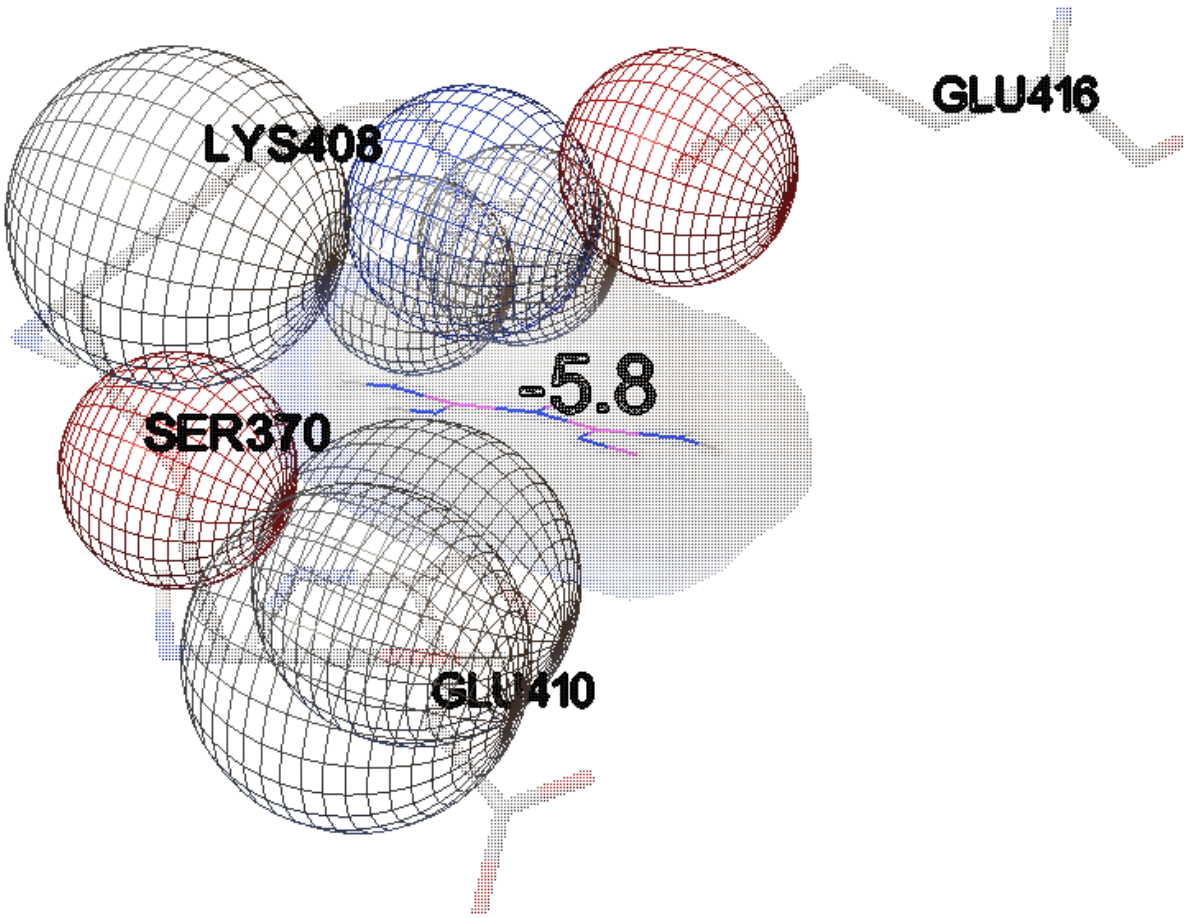


Fig 6.6: Docking of 1-Carbamimidoyl-1,2-dimethylguanidine (Similar compound of metformin, having highest binding energy)

Table 6.11: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with Berberine.

Binding modes	Affinity (kcal/mol)
1	-12.3
2	-11.7
3	-11.3

Table 6.12: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with compounds similar to BERBERINE

Compound Name	Binding energy (kcal/mol)
BERBERRUBINE	-15.4
COPTISINE	-14.7
PSEUDOBERBERINE	-14.5
THALIDENDINE	-14.5
12-Bromoberberine Chloride	-14.3
9-O-OCTYL-BERBERINE	-14.3
DEHYDROCAVIDINE	-13.6

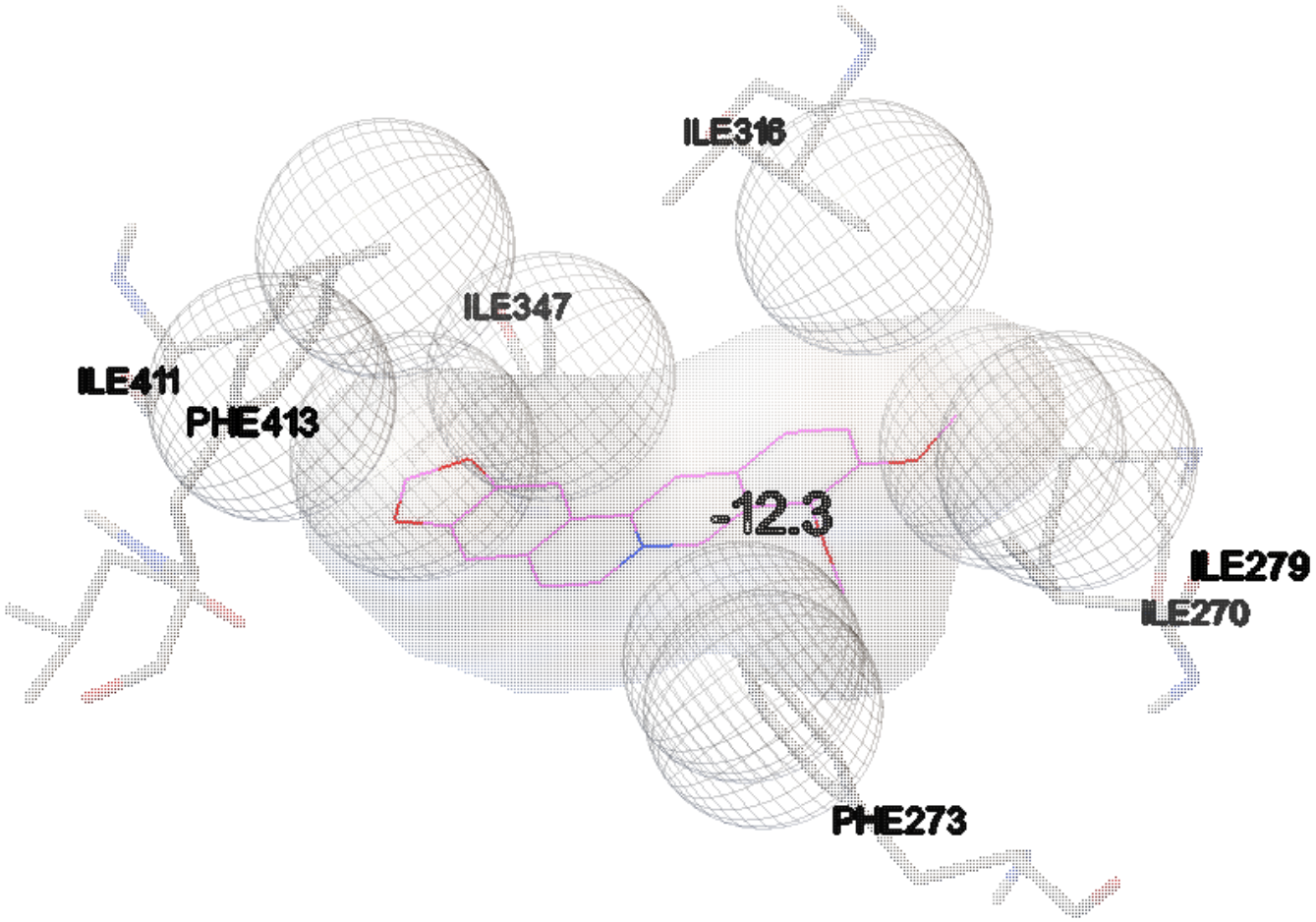


Fig 6.7: Docking of the SIRT1 protein (PDB ID:4I5I) with berberine

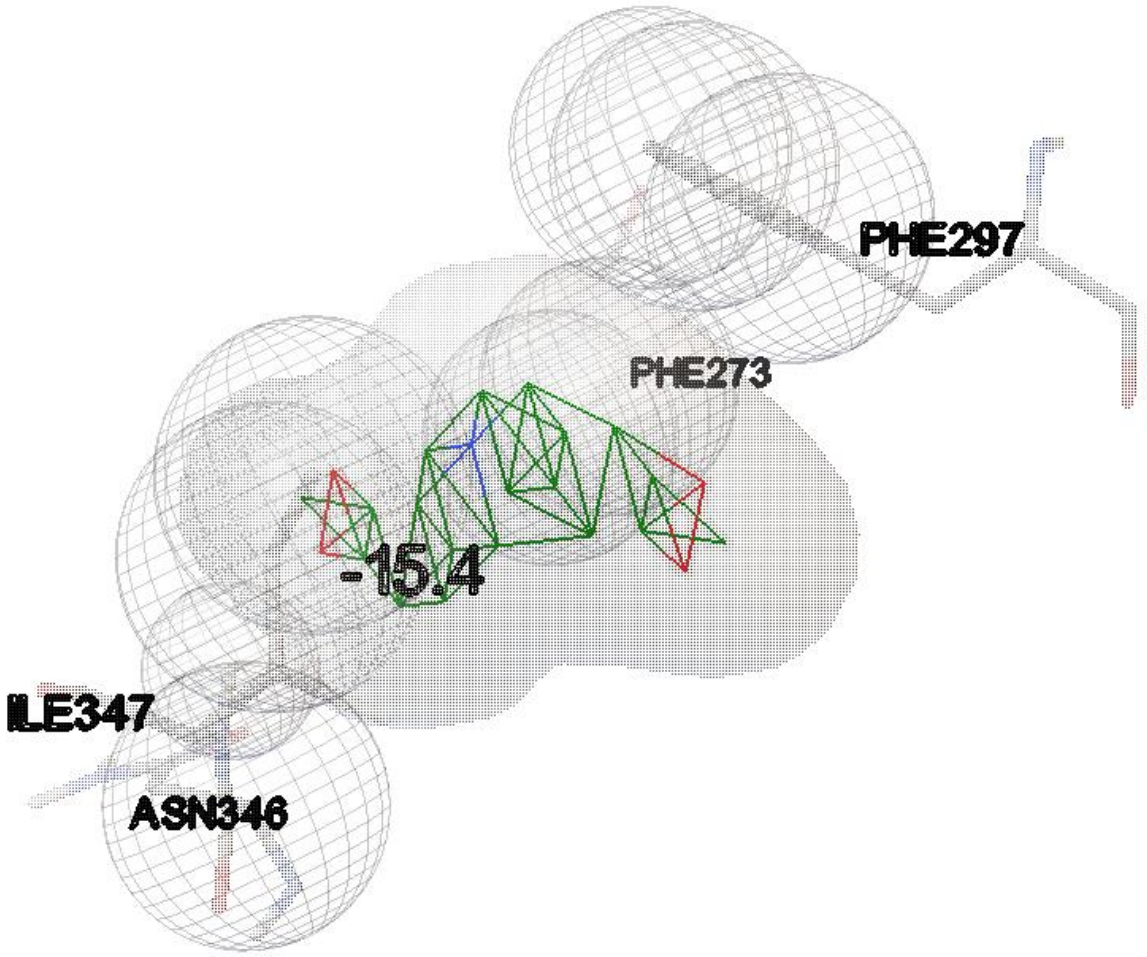


Fig 6.8: Docking of SIRT1 protein (PDB ID:4I5I) with Berberrubine (a similar compound of Berberine, having the highest binding energy)

Table 6.13: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with Esculetin.

Binding modes	Affinity (kcal/mol)
1	-9.2
2	-8.3
3	-8.3
4	-7.4
5	-7.3
6	-7.1
7	-7.1
9	-6.9
10	-6.9

Table 6.14: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with compounds similar to Esculetin

Compound Name	Binding energy (kcal/mol)
CITROPTEN	-11.3
ETHOXYCOUMARIN	-9.5
DEMETHYLSUBEROSIN	-9.3
4-METHYLESCULETIN	-9.2
ISOSCOPOLETIN	-9.2
UMB	-9.1
SCOPOLETIN	-9.1
SCOPARONE	-9.0
FRAXINOL	-9.0
HERNIARIN	-8.9
LEPTODACTYLONE	-8.9
FRAXETIN	-8.7
6-HYDROXYCOUMARIN	-8.7

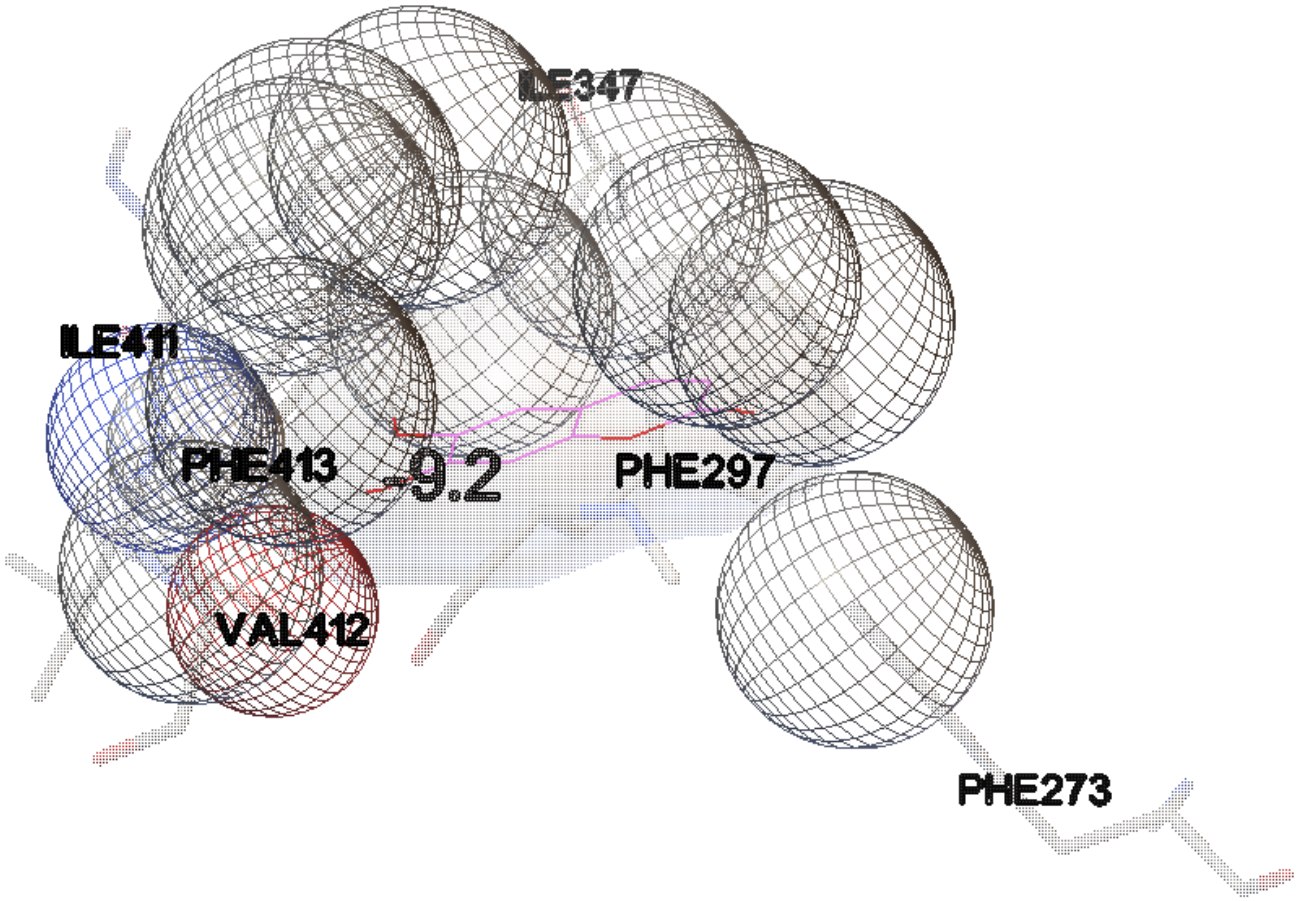


Fig 6.9: Docking of the SIRT1 protein (PDB ID:4I5I) with esculetin

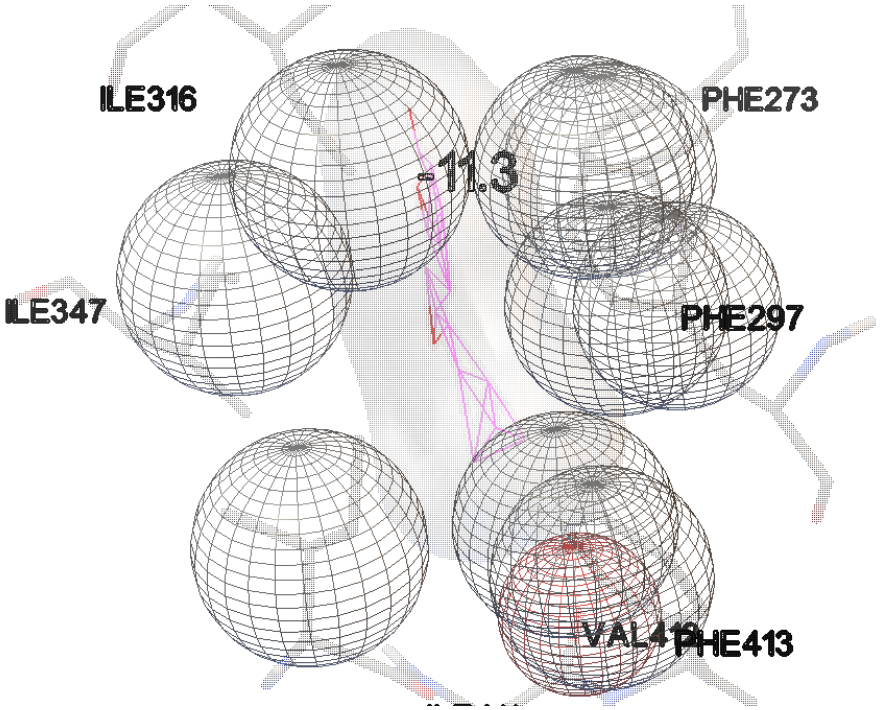


Fig 6.10: Docking of Citropten with SIRT1 protein (PDB ID:4I5I) (Similar compound of esculetin, having highest binding energy)

Table 6.15: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with Vanillic acid.

Binding modes	Affinity (kcal/mol)
1	-11.9
2	-10.9
3	-10.7
4	-9.5
5	-9.3

Table 6.16: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with compounds similar to Vanillic Acid

Compound Name	Binding energy (kcal/mol)
ISOVANILLIC ACID METHYL ESTER	-7.6
ISOVANILLIC ACID	-7.5
VERATRIC ACID	-7.3
METHYL PROTOCATECHUATE	-7.3
METHYL VANILLATE	-7.3

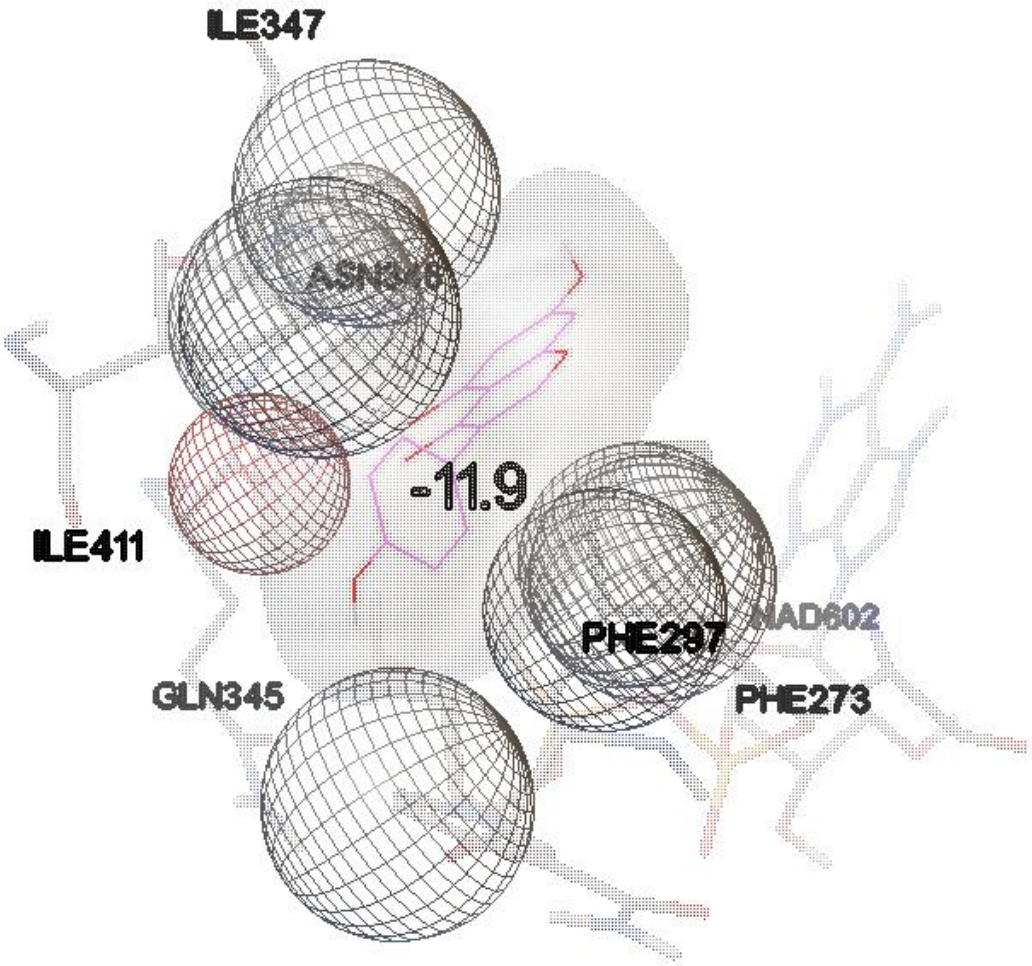


Fig 6.11: Docking of the SIRT1 protein (PDB ID:4I5I) with vanillic acid

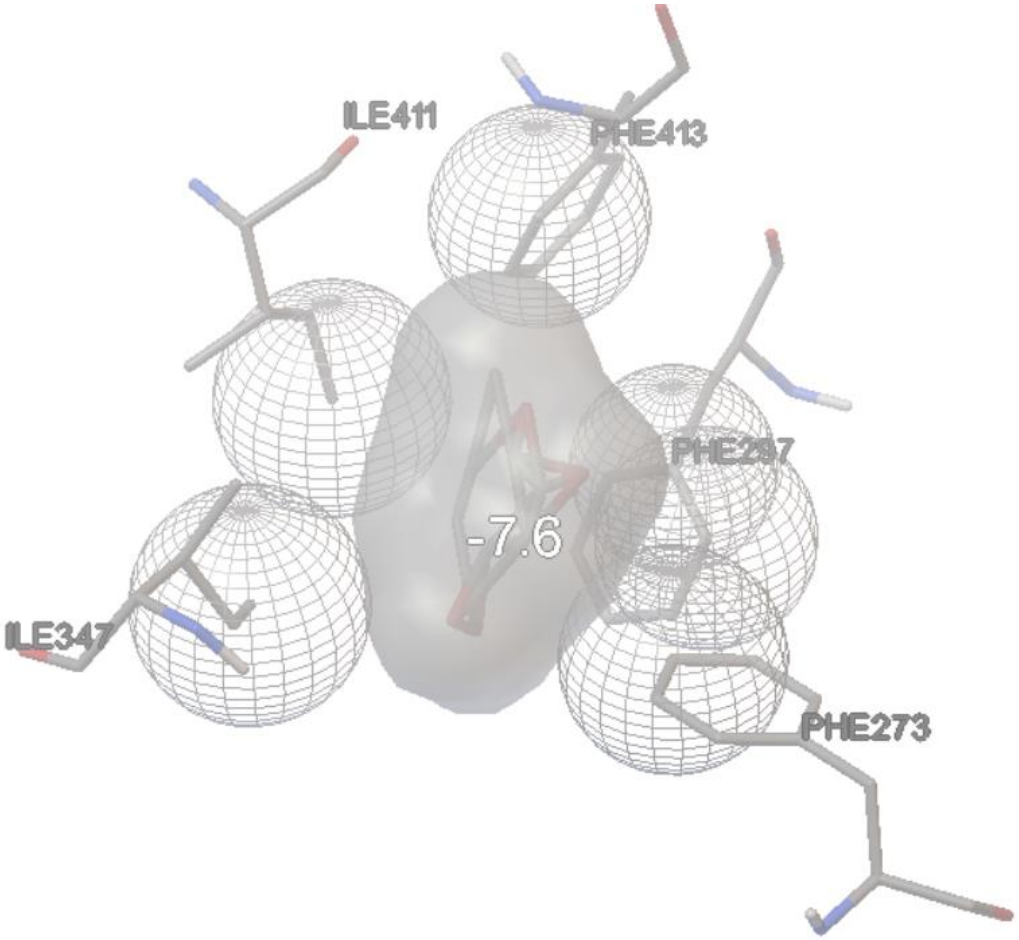


Fig 6.12 (a): Docking of Isovanillic acid Methyl Ester with SIRT1 protein (PDB ID:4I5I) (Similar compound of Vanillic acid, having highest binding energy)

Table 6.17: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with Genistein.

Binding modes	Affinity (kcal/mol)
1	-11.9
2	-10.9
3	-10.7
4	-9.5
5	-9.3

Table 6.18: Binding poses and energy when SIRT1 protein (PDB ID:4I5I) docked with compounds similar to Genistein

Compound Name	Binding energy (kcal/mol)
3'-formylgenistein	-12.9
IRISTECTORIGENIN A	-12.9
PRUNETIN	-12.9
PRATENSEIN	-12.8
ERIOPHORIN C	-12.7
2'-Hydroxygenistein	-12.6
6-Methylgenistein	-12.6
OROBOL	-12.5
IRILIN D	-12.4
THERALIN	-12.0
XENOGNOSIN B	-12.0
TECTORIGENIN	-11.8
CAJANIN	-11.5

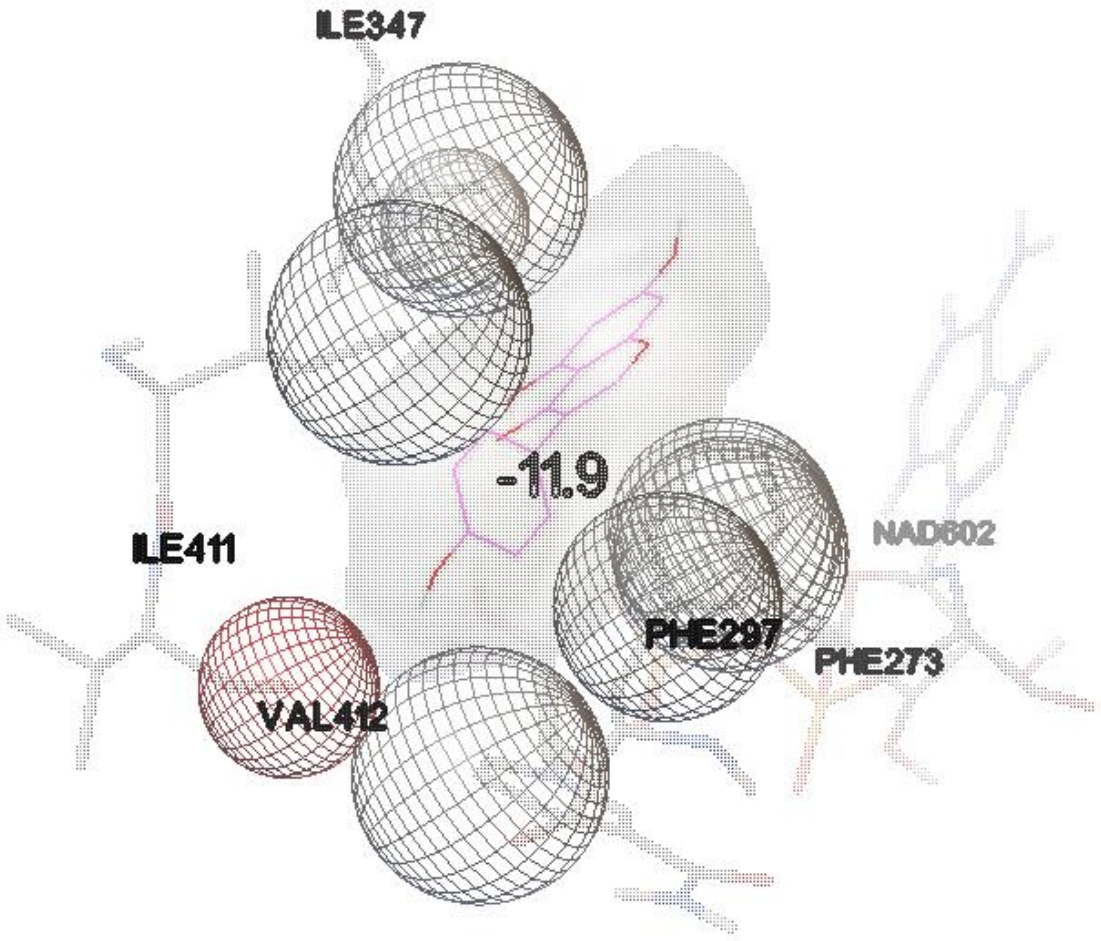


Fig 6.12 (b): Docking of the SIRT1 protein (PDB ID:4I5I) with genistein

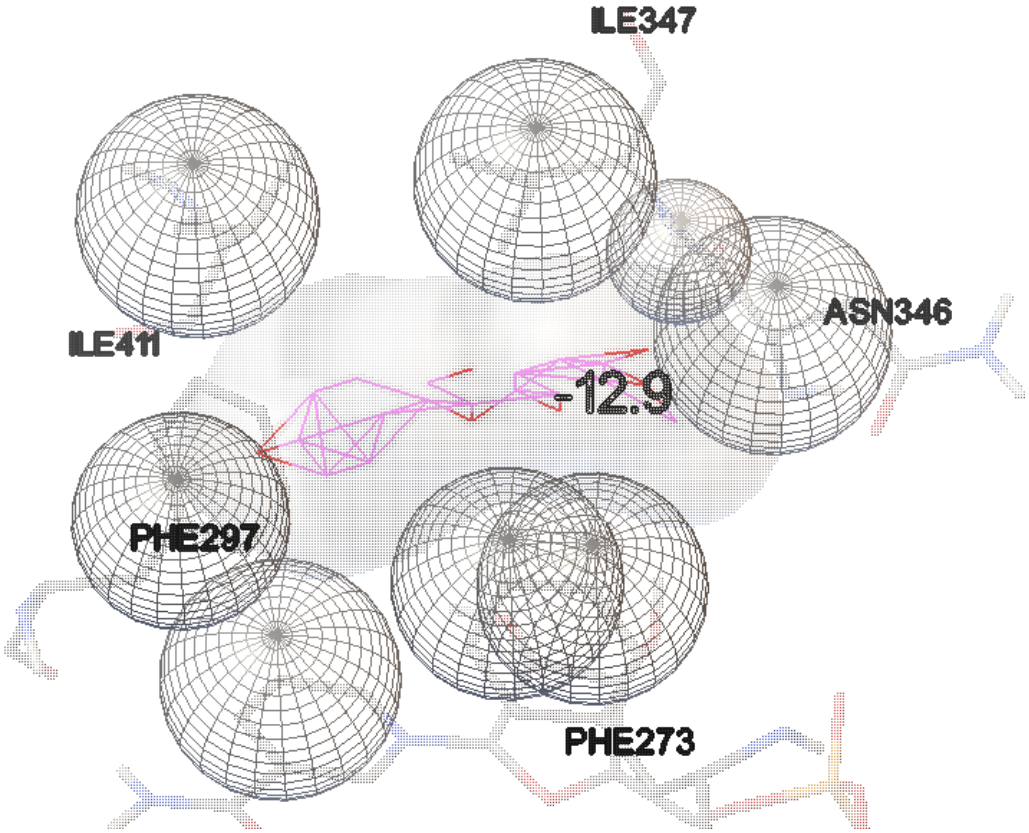


Fig 6.13: Docking of Prunetin with SIRT1 protein (PDB ID:4I5I) (Similar compound of Genistein, having highest binding energy)

6.3 In-silico ADME prediction

We have used the SwissADME web tool which is a predictive model for pharmacokinetics, drug-likeness, and physicochemical property.

When comparing the molecular weight **coptisine** had the highest molecular weight of 320.32g/mol while **4-amino butanoic acid** had the lowest molecular weight of 103.12g/mol. **4-amino butanoic acid** was found to be highly soluble in water while **coptisine** was least soluble. **4-amino butanoic acid** was found to have high GI absorption and it was not found to inhibit any of the CYP isoforms.

Based on structural similarity searching, docking, ADME studies, and keeping the economy and cost as a factor we chose **4-amino butanoic acid** and **UMB** for further in vitro and in vivo analysis.

6.4 ADME studies:

Table 6.19: Physicochemical property

Phytochemical	Molecular Weight (g/mol)	Number of heavy atoms	Number of aromatic heavy atoms	Number of H-bond acceptors	Number of H-bond donors	Molar refractivity
Pinosylvin	212.24	16	12	2	2	65.86
Cis-pinosylvin	212.24	16	12	2	2	65.86
Resveratrol 4'-methyl ether	242.27	18	12	3	2	72.35
Thalidendine	322.23	24	16	4	1	90.41
Berruberine	357.79	25	16	4	1	96.26
Psuedoberrberine	371.81	26	16	4	0	100.73
Dehydrocavidine	348.37	26	18	4	0	100.76
Coptisine	320.32	24	16	4	0	40.80
Methyl protocatechuate	168.15	16	6	4	2	66.76
Veratric acid	182.17	13	6	4	1	46.39
Isovanillic acid	168.15	12	6	4	2	41.92
Prunetin	284.26	21	16	5	2	78.46
2'-hydroxygenistein	286.24	21	16	6	4	76.01
3 formylgenistein	298.25	22	16	6	3	79.38
6-methylgenistein	284.26	21	16	5	3	90.90
Theralin	300.26	22	16	6	3	80.48
Orobol	286.24	21	16	6	4	76.01

Tectorigenin	300.26	22	16	6	3	80.48
Fraxetin	208.17	15	10	5	2	53.02
Scoparone	206.19	15	10	4	0	55.47
Citropten	206.19	15	10	4	0	55.47
Scopoletin	192.17	14	10	4	1	51
Ethoxycoumarin	190.20	14	10	3	0	53.78
Herniarin	176.17	13	10	3	0	48.98
UMB	162.14	12	10	3	1	44.51
6-hydroxycoumarin	162.14	12	10	3	1	44.51
4-aminobutanoic acid	103.12	7	0	3	2	25.82

Table 6.20: Water solubility

Phytochemical	Log S (ESOL)	Solubility	Class
Pinosylvin	-3.77	3.59e-02mg/ml	Soluble
Cis-pinosylvin	-3.77	3.59e-02mg/ml	Soluble
Resveratrol 4'-methyl ether	-3.1	3.74e-01mg/ml	Soluble
Thalidendine	-4.34	1.48e-02mg/ml	Moderately soluble
Berruberine	-5.04	3.24e-03mg/ml	Moderately soluble
Psuedoberrberine	-5.25	2.08e-03mg/ml	Moderately soluble
Dehydrocavidine	-5.81	5.43e-04mg/ml	Moderately Soluble
Coptisine	-4.52	9.72e-03 mg/ml	Moderately soluble
Isovanillic acid	-2.02	1.60+00 mg/ml	Soluble
Veratric acid	-2.13	1.36+00 mg/ml	Soluble
Methyl protocatechuate	-2.51	1.49e+00mg/ml	Soluble
Prunetin	-3.92	3.43e-02mg/ml	Soluble
2'-hydroxygenistein	-3.57	7.74e-02mg/ml	Soluble
3-formylgenistein	-3.78	4.97e-02mg/ml	Soluble
6-methylgenistein	-4.01	2.78e-02mg/ml	Moderately soluble
Theralin	-3.77	5.09e-02mg/ml	Soluble
Orobol	-3.71	5.63e-02mg/ml	Soluble
Tectorigenin	-3.77	5.09e-02mg/ml	Soluble
4-aminobutanoic acid	1.72	5.36e+03 mg/ml	Highly soluble
6-hydroxycoumarin	-2.11	1.26e+00 mg/ml	Soluble

Fraxetin	-2.30	1.06+00 mg/ml	Soluble
UMB	-2.46	5.66-01 mg/ml	Soluble
Scopoletin	-2.46	6.70e-01 mg/ml	Soluble
Herniarin	-2.53	5.18e-01 mg/ml	Soluble
Scoparone	-2.56	5.72e-01 mg/ml	Soluble
Ethoxycoumarin	-2.97	2.06e-01 mg/ml	Soluble

Table 6.21: Pharmacokinetic Property

Phytochemical	GI Absorption	BBB Permeant	P-gp substrate	CYP1A2 Inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	Log K_pSkin Permeation (cm/s)
Pinosylvin	High	Yes	No	Yes	No	Yes	-5.12
Cis-pinosylvin	High	Yes	No	Yes	No	Yes	-5.12
Resveratrol 4'-methyl ether	High	Yes	No	Yes	No	Yes	-5.33
Thalidendine	High	Yes	Yes	Yes	No	No	-5.93
Berruberine	High	No	Yes	Yes	No	No	-5.58
Psuedoberrberine	High	No	Yes	Yes	No	No	-5.43
Dehydrocavidine	High	Yes	Yes	Yes	Yes	Yes	-4.6
Coptisine	High	Yes	Yes	Yes	No	No	-5.78
Methyl protocatechuate	High	No	No	No	No	No	-6.27
Isovanillic acid	High	No	No	No	No	No	-6.31
Veratric acid	High	Yes	No	No	No	No	-6.27
Prunetin	High	No	No	Yes	No	No	-5.91
2'-hydroxygenistein	High	No	No	Yes	No	No	-6.41
3-formylgenistein	High	No	No	No	No	No	-6.22
6-methylgenistein	High	No	No	Yes	No	No	-5.88
Theralin	High	No	No	Yes	No	No	-6.26
Orobol	High	No	No	Yes	No	No	-6.25
Tectorigenin	High	No	No	Yes	No	No	-6.25
4-aminobutanoic acid	High	No	No	No	No	No	-9.18

Herniarin	High	Yes	No	Yes	No	No	-6.14
UMB	High	Yes	No	Yes	No	No	-6.17
Fraxetin	High	No	No	No	No	No	-6.74
Scopoletin	High	Yes	No	Yes	No	No	-6.39
Scoparone	High	Yes	No	Yes	No	No	-6.34
Ethoxycoumarin	High	Yes	No	Yes	No	No	-5.71
6-hydroxycoumarin	High	Yes	No	Yes	No	No	-6.56

Table 6.22: Druglikeness

Phytochemical	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Pinosylvin	Yes	Yes	Yes	Yes	Yes	0.55
Cis-pinosylvin	Yes	Yes	Yes	Yes	Yes	0.55
Resveratrol 4'-methyl ether	Yes	Yes	Yes	Yes	Yes	0.55
Thalidendine	Yes	Yes	Yes	Yes	Yes	0.55
Berruberine	Yes	Yes	Yes	Yes	Yes	0.55
Psuedoberrberine	Yes	Yes	Yes	Yes	Yes	0.55
Dehydrocavidine	Yes	Yes	Yes	Yes	No	0.55
Coptisine	Yes	Yes	Yes	Yes	Yes	0.55
Methyl protocatechuate	Yes	Yes	Yes	Yes	No	0.55
Isovanillic acid	Yes	Yes	Yes	Yes	No	0.56
Veratric acid	Yes	Yes	Yes	Yes	No	0.56
Prunetin	Yes	Yes	Yes	Yes	Yes	0.55
2'-hydroxygenistein	Yes	Yes	Yes	Yes	Yes	0.55
3 formylgenistein	Yes	Yes	Yes	Yes	Yes	0.55
6-methylgenistein	Yes	Yes	Yes	Yes	Yes	0.55
Theralin	Yes	Yes	Yes	Yes	Yes	0.55
Orobol	Yes	Yes	Yes	Yes	Yes	0.55
Tectorigenin	Yes	Yes	Yes	Yes	Yes	0.55

4-aminobutanoic acid	Yes	No	Yes	Yes	No	0.55
Herniarin	Yes	Yes	Yes	Yes	No	0.55
UMB	Yes	No	Yes	Yes	No	0.55
Fraxetin	Yes	Yes	Yes	Yes	Yes	0.55
Scopoletin	Yes	Yes	Yes	Yes	Yes	0.55
Scoparone	Yes	Yes	Yes	Yes	Yes	0.55
Ethoxycoumarin	Yes	Yes	Yes	Yes	No	0.55
6-hydroxycoumarin	Yes	No	Yes	Yes	No	0.55
Citropten	Yes	Yes	Yes	Yes	Yes	0.55

Table 6.23: Medicinal chemistry

Phytochemical	PAINS	BRENK	LEADLIKENESS	SYNTHETIC ACCESSIBILITY
Pinosylvin	0	1	No	1.98
Cis-pinosylvin	0	1	No	1.98
Resveratrol 4'-methyl ether	0	1	No	2.08
Thalidendine	0	1	Yes	3.02
Berruberine	0	1	No	3.05
Psuedoberrberine	0	1	No	3.12
Dehydrocavidine	1	3	No	2.64
Coptisine	0	1	YES	2.96
Methyl protocatechuate	1	1	No	1.37
Isovanillic acid	0	0	NO	1.24
Veratric acid	0	0	NO	1.55
Prunetin	0	0	YES	3.03
2'-hydroxygenistein	1	1	YES	2.99
3 formylgenistein	0	0	YES	3.04
6-methylgenistein	0	0	YES	2.96
Theralin	0	1	YES	3.01
Orobol	0	0	YES	2.95
Tectorigenin	0	0	YES	2.96

4-aminobutanoic acid	0	0	NO	1.00
Herniarin	0	1	NO	2.59
UMB	0	1	NO	2.56
Fraxetin	1	2	NO	2.87
Scopoletin	0	1	NO	2.62
Scoparone	0	1	NO	2.77
Ethoxycoumarin	0	1	NO	2.74
6-hydroxycoumarin	0	1	NO	2.48
Citropten	0	1	NO	2.78

6.5 In vitro study model:

6.5.1 Total protein estimation in cells using the lowry method

Using a standard curve produced from bovine serum albumin (Figure 6.14), the protocol published by Lowry et al. (1951) was used to determine the protein content (BSA). Before beginning any investigation, the total protein content of the cell or tissue sample must be estimated. The enzyme activity is percentage of the total protein content. For the aim of separation, purification, or analysis, it is crucial to ascertain the precise activity of a given enzyme activity (Lowry et al., 1951).

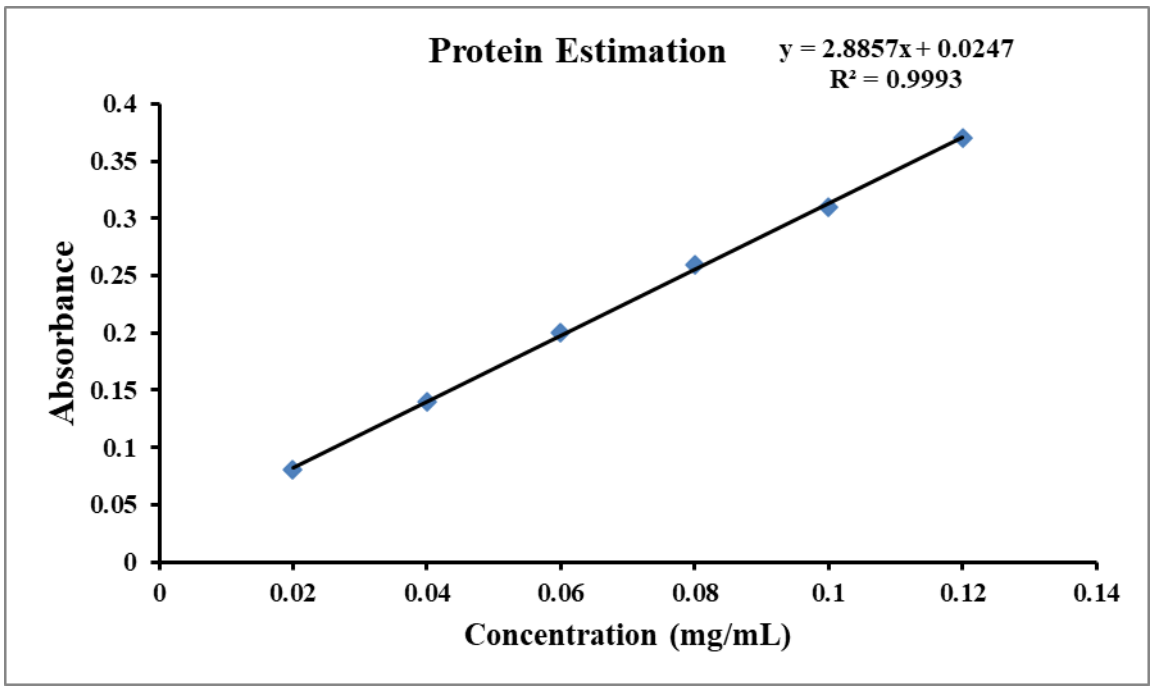


Figure 6.14: Protein estimation using the Lowry method

6.5.2 Cell line toxicity assay

We did an MTT assay on the 3T3L-1 cell line so that the effect of UMB and GABA can be checked on normal cell lines. We took concentrations of 0, 5, 25, 50, 100, or 150 µM. In pre-clinical drug research, potential drug candidates are frequently evaluated on cell lines to determine any negative effects the material may have on the cells. The results illustrate %cell viability at different concentrations. The result is presented in Table 6.24. Cells upon treatment with UMB at 5, 25, and 50µM concentration demonstrated the highest cell viability of 87.63±6.59, 81.84±5.21 and 76.46±2.47 respectively (Figure 6.15). Upon treatment with GABA cells at 5 and 25µM had the highest cell viability of 90.82±4.87 and 85.77±5.25 (Figure 6.16). The experiment was done in triplicates. The IC_{50} value for UMB was 199µM and GABA was 145µM. Negative control was DMEM media along with cells.

Table 6.24: Percentage of cell viability after GABA and UMB treatment at the different concentrations on the 3T3-L1 cell line (All the values are shown as Mean ± SEM, n=3)

The concentration of UMB (µM)	% Cell viability UMB	The concentration of GABA (µM)	% Cell viability GABA
5	87.63±6.59	5	90.82±4.87
25	81.84±5.21	25	85.77±5.25
50	76.46±2.47	50	68.35±1.32
100	70.30±0.08	100	61.40±0.47
150	67.93±4.73	150	51.39±0.03

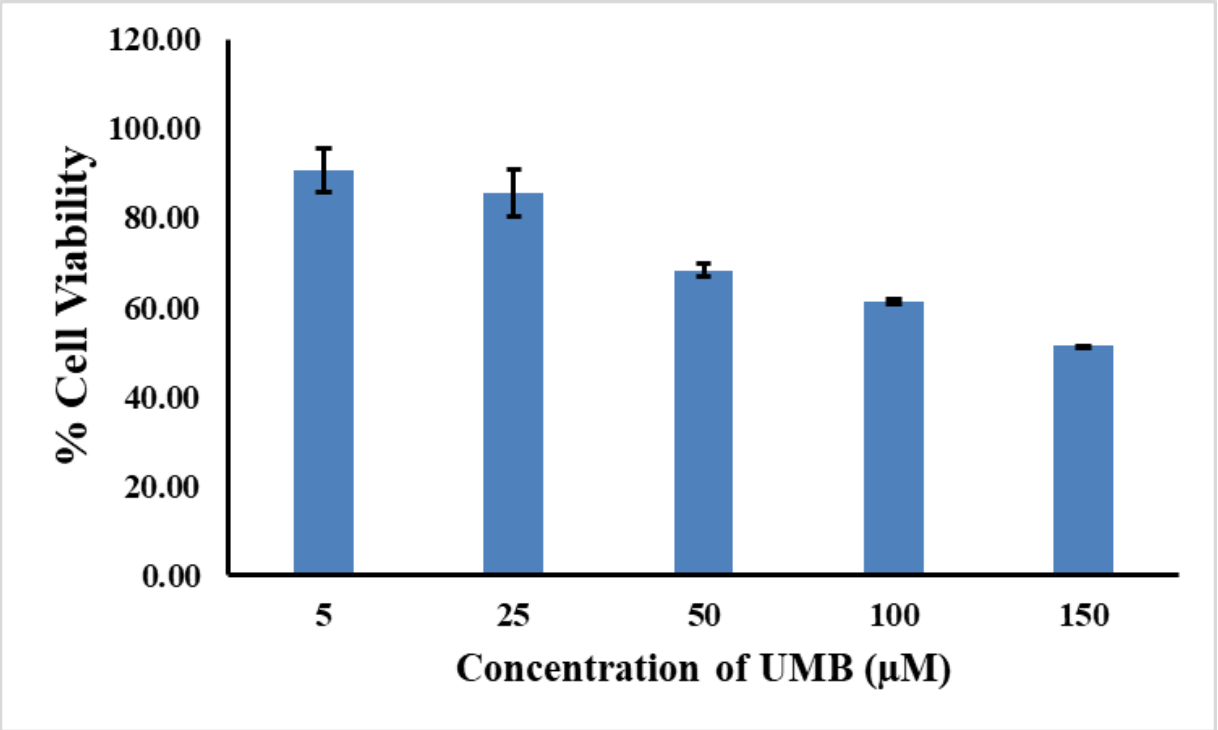


Figure 6.15: Cell line toxicity assay using 3T3L-1 cell line

(Percentage cell viability post-UMB treatment at the different concentrations on 3T3L-1 cell line, all the values are shown as Mean±SEM, n=3)

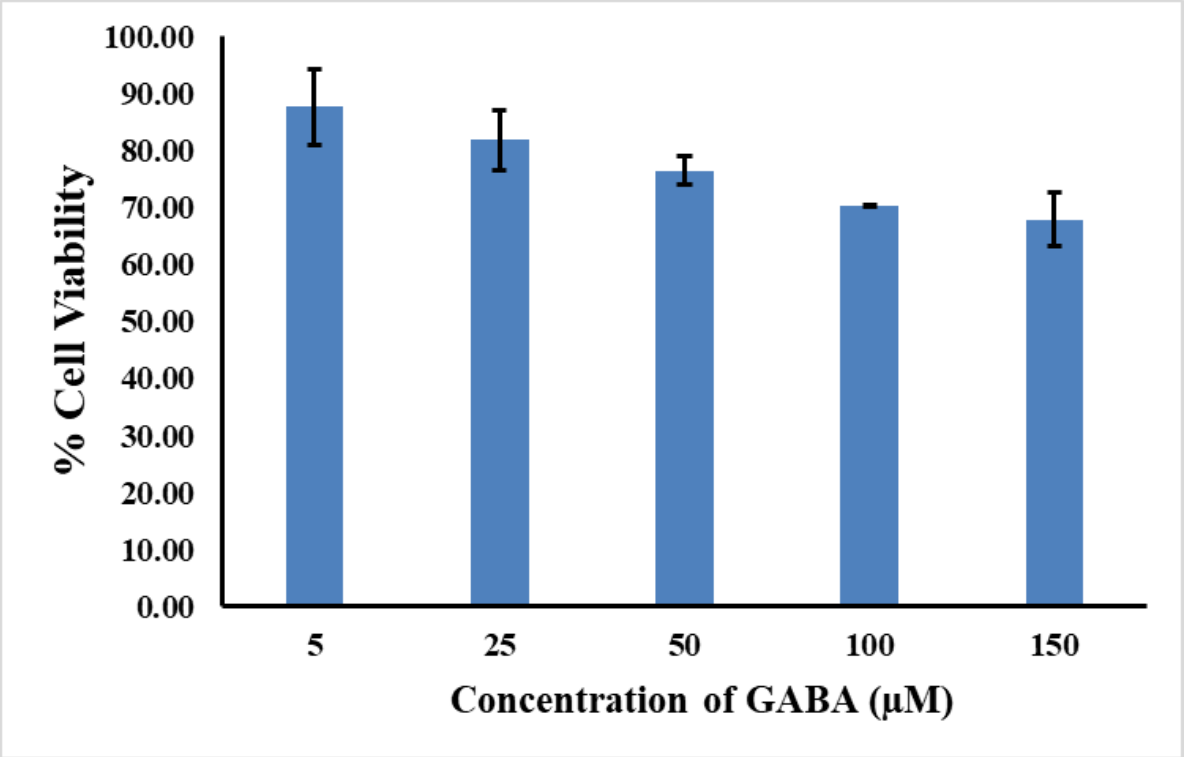


Figure 6.16: Cell line toxicity assay using 3T3L-1 cell line

(Percentage cell viability post-UMB treatment at the different concentrations on 3T3L-1 cell line, all the values are shown as Mean±SEM, n=3)

6.5.3 DPPH free radical scavenging activity

It was done to check the antioxidant potential of our selected compounds which was checked using DPPH as performed earlier by (Sharma et al., 2009). UMB was found to be a better antioxidant than GABA as it inhibited more with increasing concentration. We used ascorbic acid as the positive control. Data is shown in Table 6.25. UMB at 75µM inhibited 62% (Figure 6.17) of DPPH while GABA at 75µM inhibited only 37% (Figure 6.18) of the DPPH. Negative control was DPPH solution only without any sample.

Table 6.25: DPPH scavenging activity at different concentrations of phytochemicals

Concentration (µM)	DPPH Scavenging of UMB	DPPH scavenging of GABA
5	16.19±0.12	17.47±0.26
25	22.26±0.11	31.23±0.13
75	62.83±0.36	37.75±0.58
100	70.4±0.32	42.57±0.27

150	77.06±0.42	49.45±0.29
200	86.33±1.78	55.76±1.42
250	97±0.94	60.73±1.16

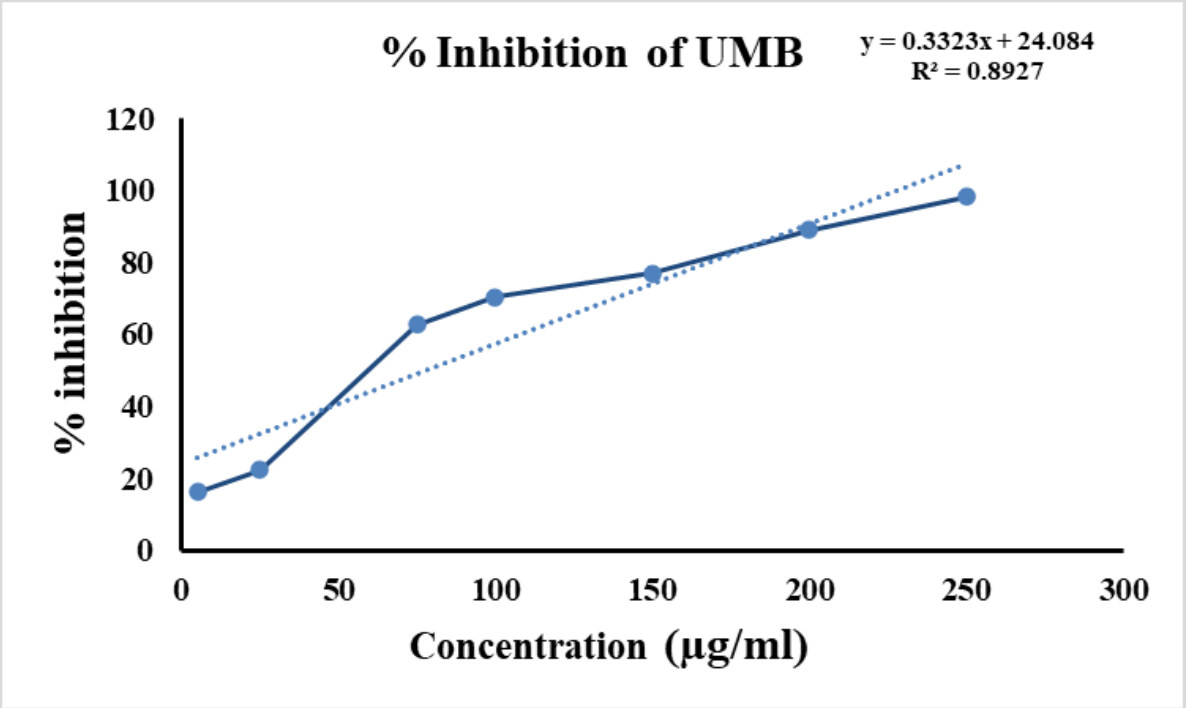


Figure 6.17: DPPH scavenging activity of UMB. The data is provided as mean±SD of 3 independent tests

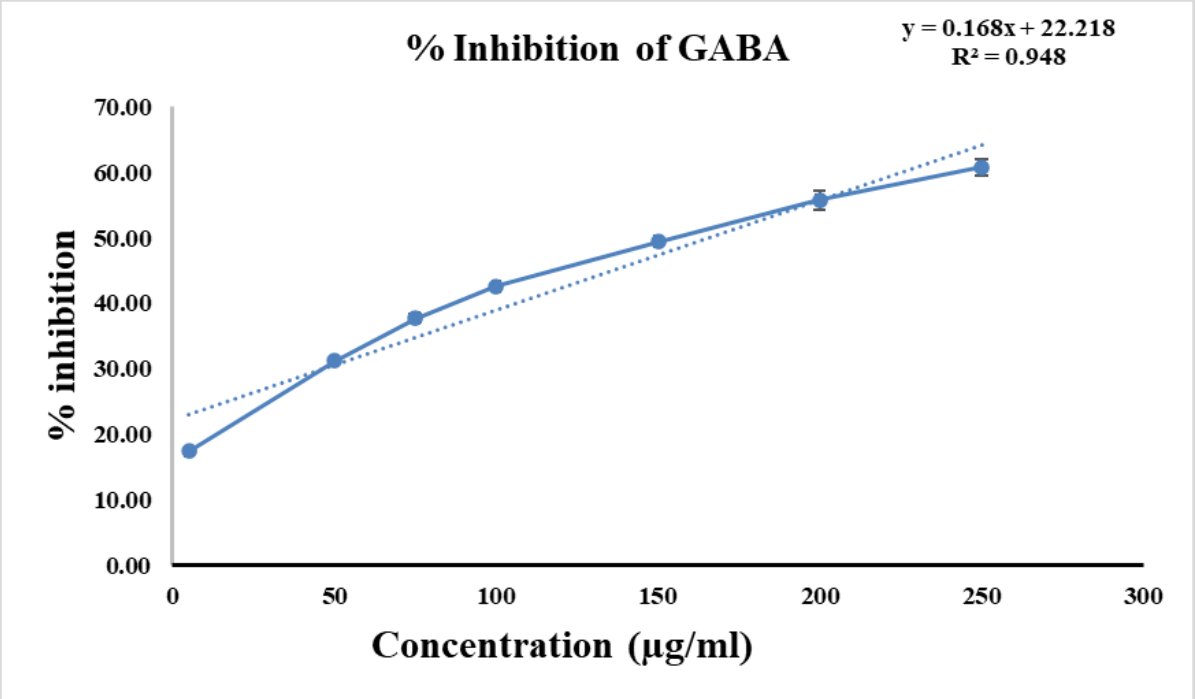
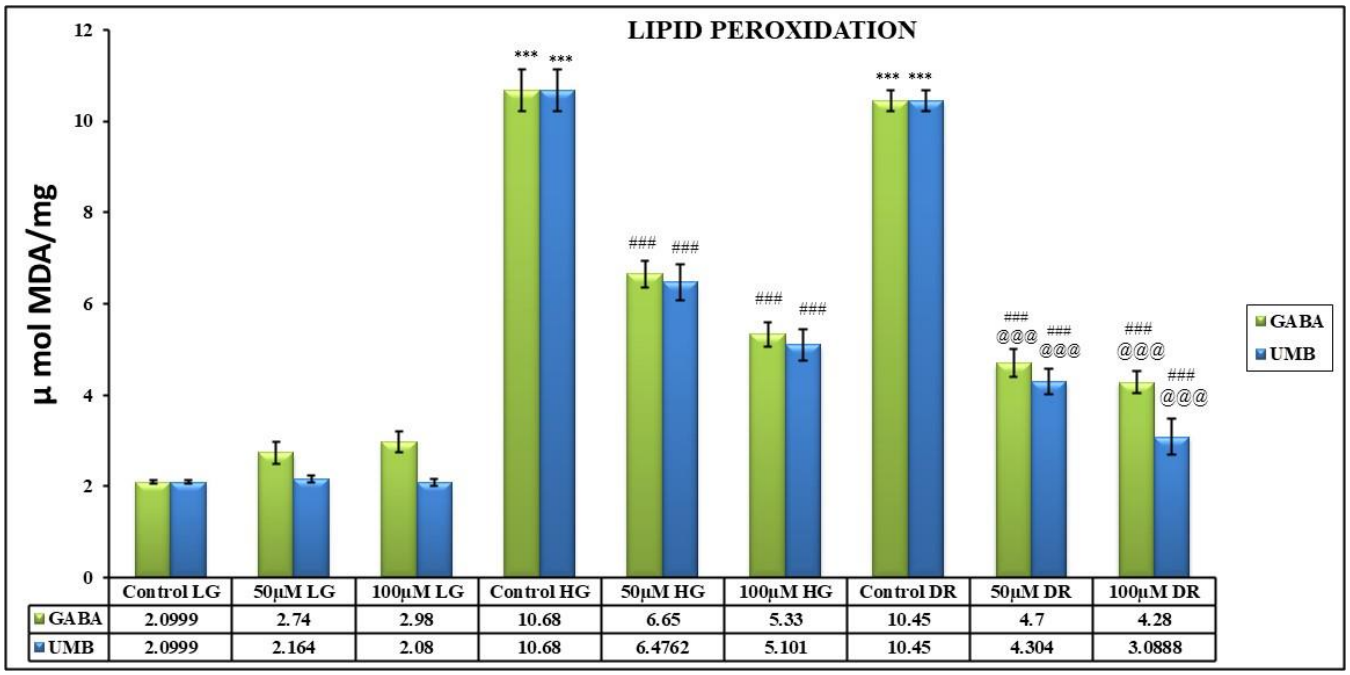


Figure 6.18: DPPH scavenging activity of GABA. The data is provided as mean±SEM of 3 independent tests

6.6 In vitro oxidative biomarkers analysis

6.6.1 Lipid Peroxidation

An indicator of fat degradation brought on by LPO is TBARS. Results showed that compared to low glucose (LG), untreated hyperglycemic/high glucose (HG) and diet reversal (DR) exhibit a high amount of TBARS. The levels of MDA were found to be elevated in control HG (10.68±0.463µmolMDA/mg) and Control DR groups (10.45±0.231µmolMDA/mg) when compared to control LG (2.09±0.04µmolMDA/mg). The MDA levels did not lower much upon administration of GABA and UMB in HG-treated cells. But when GABA and UMB were administered to the diet reversal group the MDA levels came down. The level of TBARS decreased dose-dependently when UMB and GABA were administered. 50µM GABA when given to the DR group, reduced TBARS level to 4.7±0.308 µmol MDA/mg and 100µM GABA reduced TBARS level to 4.28±0.24 µmol MDA/mg. 50µM UMB when given to the DR group, reduced the TBARS level to 4.304±0.273 µmol MDA/mg, and 100µM UMB reduced the TBARS level to 3.08±0.396 µmol MDA/mg (Figure 6.19).



	Control LG	50µM LG	100µM LG	Control HG	50µM HG	100µM HG	Control DR	50µM DR	100µM DR
GABA	2.0999	2.74	2.98	10.68	6.65	5.33	10.45	4.7	4.28
UMB	2.0999	2.164	2.08	10.68	6.4762	5.101	10.45	4.304	3.0888

Fig 6.19: Effect of GABA and UMB treatment on LPO in post-nuclear supernatant of cells after cell lysis.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control HG; ***p<0.001, **p<0.01 and *p<0.05, vs control LG; and @@@p<0.001, @@p<0.01 and @p<0.05, vs Control DR

6.6.2 GST

Results showed that compared to LG, untreated HG and DR exhibit a low level of GST enzyme. GST level was found to be decreased in control HG (7.27±0.76 U/mg)) and Control DR groups (10.41±0.267 U/mg) when compared to control LG (25.68±0.43 U/mg). The GST levels did not increase much upon administration of GABA and UMB in HG-treated cells. But when GABA and UMB were administered to the diet reversal group the GST levels came down. GST level increased dose-dependently when UMB and GABA were administered. 50µM GABA when given to the DR group, increased GST level to 17.707±0.352 U/mg and 100µM GABA increased GST level to 20.86±0.59 U/mg. 50µM UMB when given to the DR group, reduced the GST level to 18.099±0.309 U/mg and 100µM UMB increased the GST level to 20.17±1.39 U/mg) (Figure 6.20).

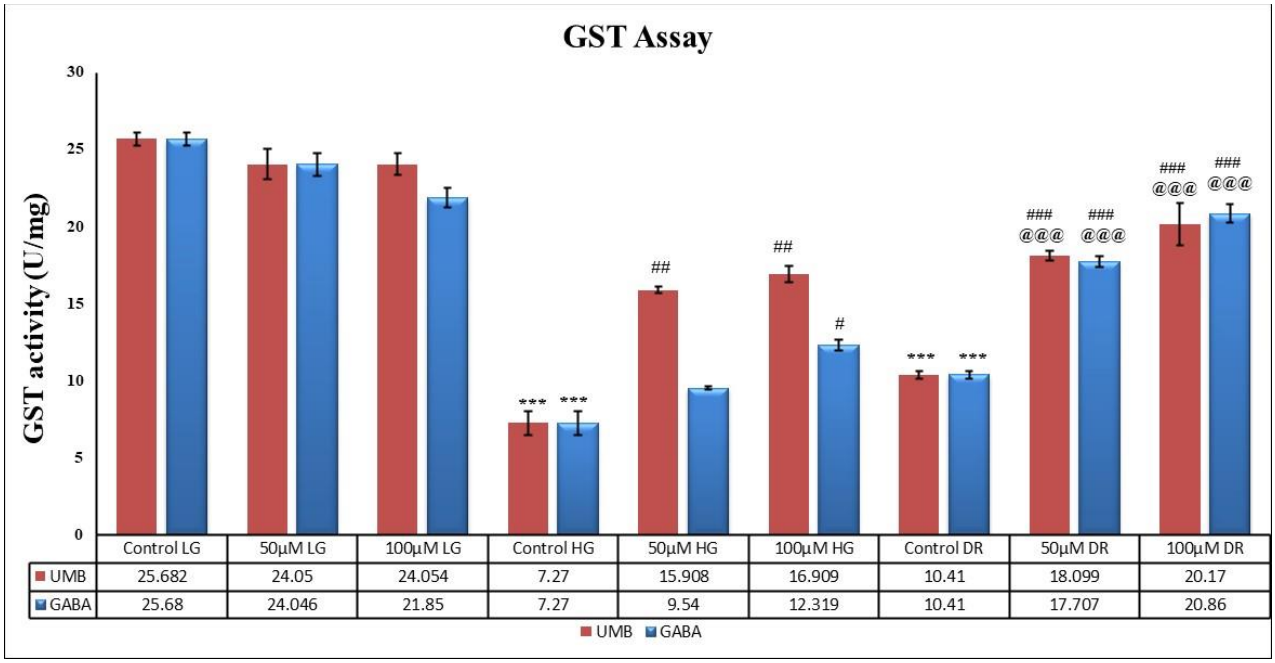


Fig 6.20: Effect of GABA and UMB treatment on GST level in post-nuclear supernatant of cells after cell lysis.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control HG; ***p<0.001, **p<0.01 and *p<0.05, vs control LG; and @@@p<0.001, @@p<0.01 and @p<0.05, vs Control DR

6.6.3 SOD

Results showed that compared to LG, untreated HG, and DR exhibit a low level of SOD enzyme. SOD level was found to be decreased in control HG (44.67±1.763 U/mg) and Control DR groups (70.93±3.937 U/mg) when compared to control LG (206.82±3.14 U/mg). The SOD levels did not increase much upon administration of GABA and UMB in HG-treated cells. But when GABA and UMB were administered to the diet reversal group the SOD levels came down. SOD level increased dose-dependently when UMB and GABA were administered. 50µM GABA when given to the DR group, increased SOD level to 143.18±2.014 U/mg, and 100µM GABA increased SOD level to 188.26±3.527 U/mg. 50µM UMB when given to the DR group, increased GST level to 145.81±0.764 U/mg and 100µM UMB increased GST level to 189.61±0.802 U/mg (Figure 6.21).

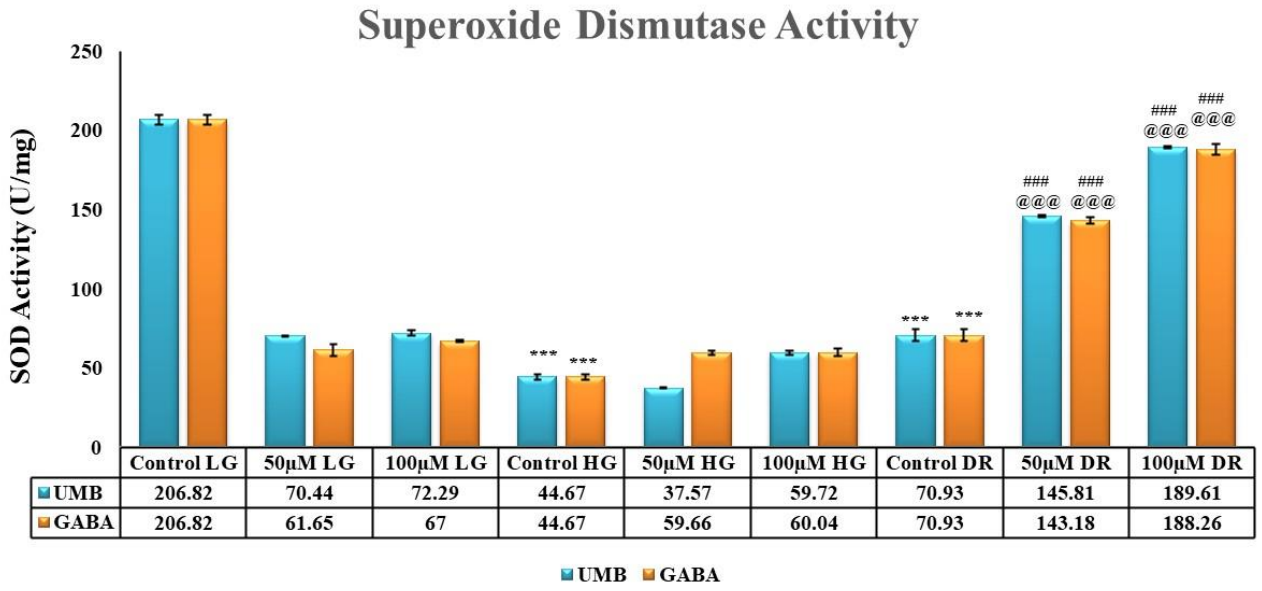


Fig 6.21: Effect of GABA and UMB treatment on SOD level in post-nuclear supernatant of cells after cell lysis.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control HG; ***p<0.001, **p<0.01 and *p<0.05, vs control LG; and @@@p<0.001, @@p<0.01 and @p<0.05, vs Control DR

6.6.4 Catalase test

Results showed that compared to low glucose (LG), untreated hyperglycemic/high glucose (HG) and diet reversal (DR) exhibit a low level of catalase enzyme. Catalase level was found to be decreased in control HG (12.36±1.0.704 U/mg) and Control DR groups (14.22±0.556 U/mg) when compared to control LG (78.65±0.36 U/mg). The catalase levels did not increase much upon administration of GABA and UMB in HG-treated cells. But when GABA and UMB were administered to the diet reversal group the catalase levels came down. Catalase level increased dose-dependently when UMB and GABA were administered. 50µM GABA when given to the DR group, increased catalase level to 65.973±0.14 U/mg, and 100µM GABA increased catalase level to 69.125±2.17 U/mg. 50µM UMB when given to the DR group, increased catalase level to 62.094±0.554 U/mg and 100µM UMB increased catalase level to 72±1.12 U/mg (Figure 6.22).

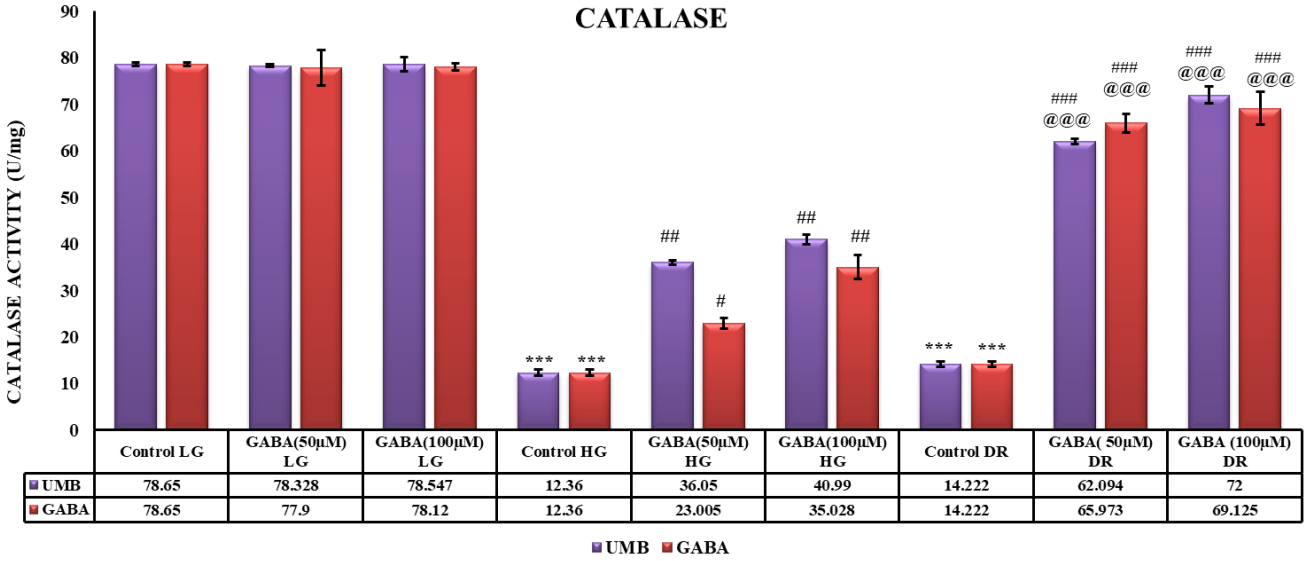


Fig 6.22: Effect of GABA and UMB treatment on catalase level in post-nuclear supernatant of cells after cell lysis.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control HG; ***p<0.001, **p<0.01 and *p<0.05, vs control LG; and @@@p<0.001, @@p<0.01 and @p<0.05, vs Control DR

6.7 In vivo studies

Since in vitro analysis proved the compounds were a good antioxidant agent and were able to ameliorate GM. So, to check its efficacy in in vivo conditions we performed an analysis on SDrats. 102 rats were divided into 15 groups, where for the first 16 weeks rats were segregated into 2 groups of NPD fed and HFD fed, and then for the next 8 weeks, the diets were reversed.

6.7.1 Body weight estimation

The body weights (g) of the rat were evaluated on the first day before the introduction of HFD to the rat. The body weight of the rat did not alter much on the first day. After 16 weeks of feeding HFD, we noted increased body weight of the rat in HFD Groups 4-15 when compared to the NPD control group. After eight weeks of diet reversal, we observed a drop in the rats' body weight in groups 10-15 but no decrease in weight was observed in groups 4-9 (Figure 6.23 and Table 6.26).

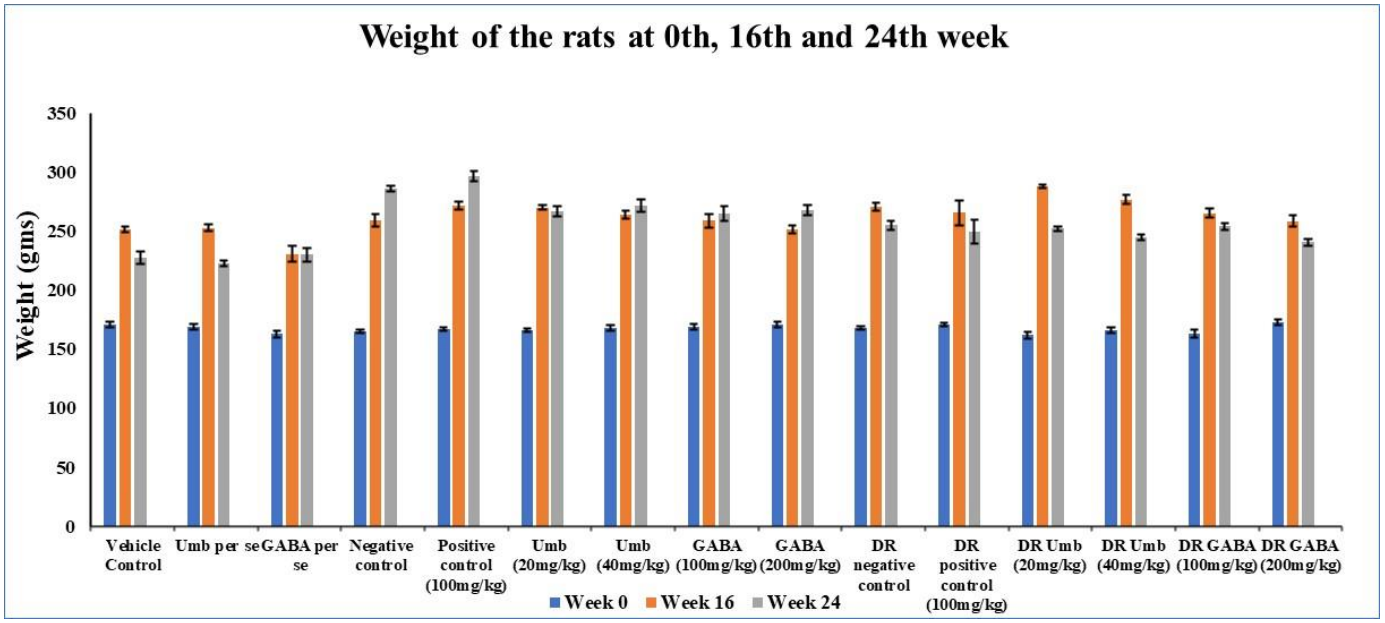


Figure 6.23: Effect of GABA and UMB treatment on body weight of the rats, post-treatment. All the results are presented as Mean±SEM where n=6 for vehicle control, UMB *per se*, and GABA *per se*, and n=7 for the rest of the groups.

Table 6.26: Effect of different treatments on body weight (g±SEM) of rats in different groups

Groups	Week 0	SEM	Week 16	SEM	Week 24	SEM
Vehicle Control	171	2.45	251.33	2.56	227.33	5.45
UMB per se	169	2.17	252.83	2.45	222.5	2.4
GABA per se	163	3.1	230.5	6.66	230	5.56
Neg control	165	1.69	259.25	5.57	286	2.577
Pos control (100mg/kg)	167	1.16	271.5	3.53	296.5	4.44
UMB (20mg/kg)	166	1.68	270	2.05	267	4.08
UMB (40mg/kg)	168	2.83	264	3.01	271.66	4.95
GABA (100mg/kg)	169	1.92	258.75	5.58	265	6.02
GABA (200mg/kg)	171	2.22	251.5	3.09	268	4.21
DR neg control	168	1.59	270.75	3.54	255	4.1
DR pos control (100mg/kg)	171	1.68	265.5	10.779	249.75	10.05
DR UMB (20mg/kg)	162	2.88	288	1.48	252	1.83
DR UMB (40mg/kg)	166	2.14	276.75	4.12	244.75	2.769

DR GABA (100mg/kg)	163	3.55	265	3.95	254	2.84
DR GABA (200mg/kg)	173	2.7	258.5	4.9	240.75	2.84

6.7.2 Blood Plasma Glucose levels:

Blood glucose level was tested after the 16th week of the study and the NPD group had a blood glucose level of 122±2.12mg/dL while the HFD group after 16 weeks of HFD feeding had a blood glucose level of 132±1.76mg/dL (Figure 6.24). The Vehicle Control group had a blood glucose level of 110.8±0.43mg/dL while the negative control group had a blood glucose level of 137±0.40mg/dL. Upon treatment with metformin, blood glucose level decreased to 107±0.14mg/dL but treatment with UMB and GABA did not reduce blood glucose levels much. But upon diet reversal and with the treatment of phytochemicals, the blood glucose level was normal as compared to the vehicle control group. The UMB and GABA brought the blood glucose level down as a high dose of UMB in the DR group decreased the blood glucose level to 116.2±0.2mg/dL and a high dose of GABA in the diet Reversal group decreased the blood glucose level to 128.57±0.08mg/dL (Figure 6.25).

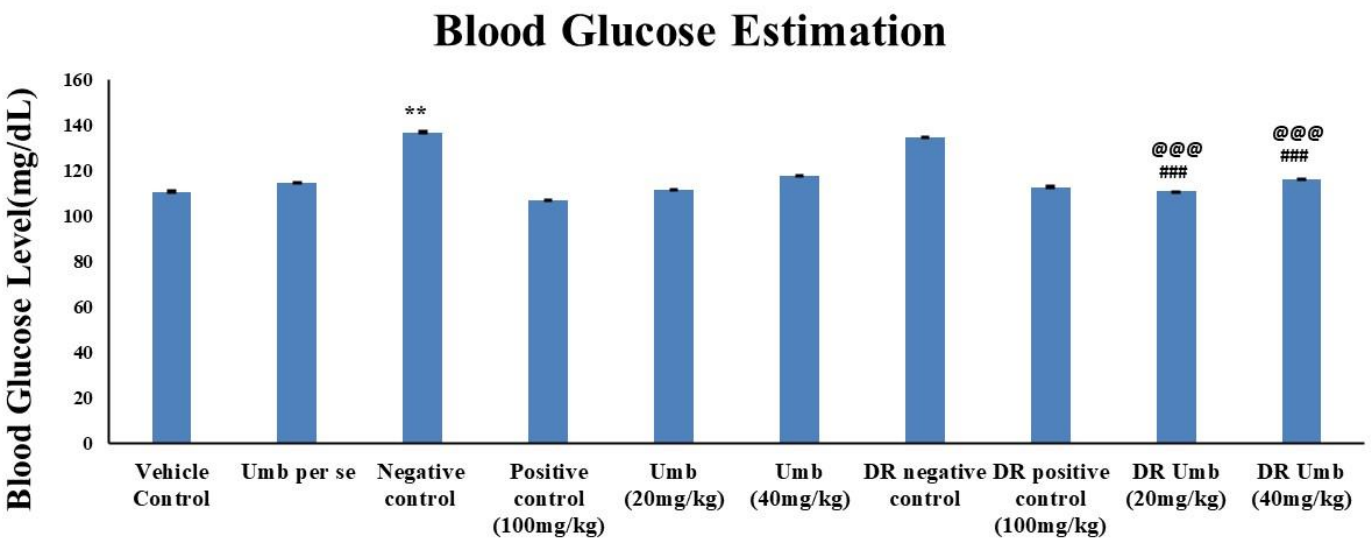


Figure 6.24: Effect of UMB treatment on blood glucose levels after the study. All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

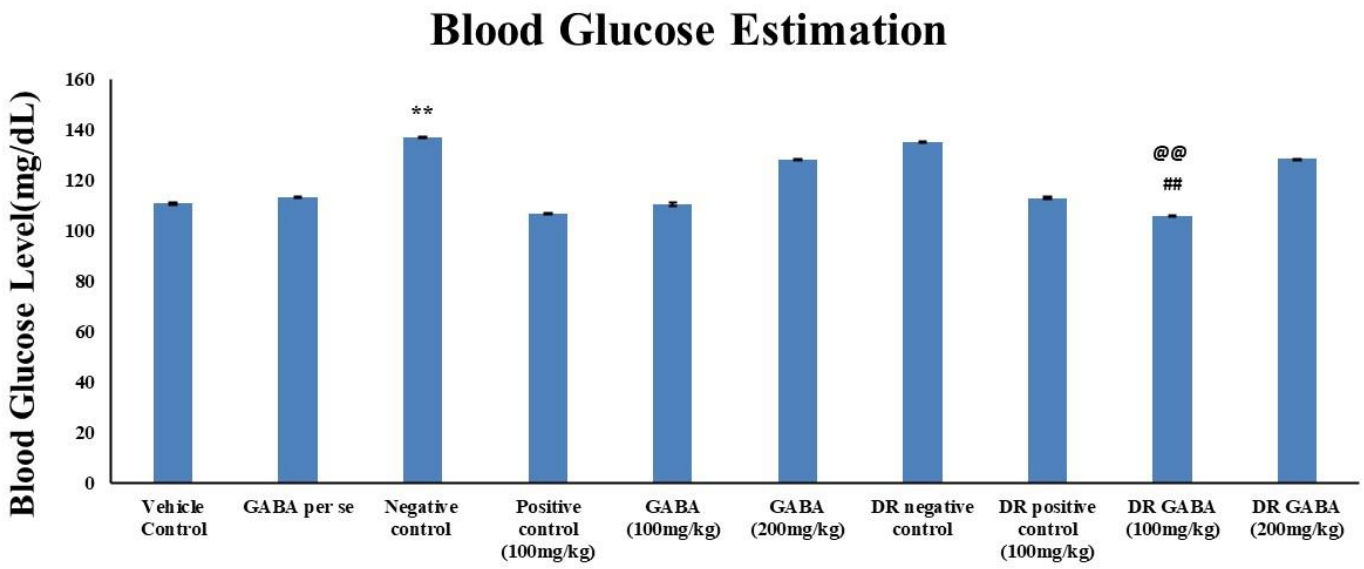


Figure 6.25: Effect of GABA treatment on blood glucose levels after the study. All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.7.3 Biochemical Parameters

6.7.3.1 Lipid Profile Estimation

LDL, Cholesterol, HDL, Triglyceride, and VLDL were tested in the blood serum of overnight fasting animals. Cholesterol (201±0.1mg/dL) (Figure 6.26), triglyceride (179±2.79mg/dL) (Figure 6.28), and LDL (86.2±0.1mg/dL) levels (Figure 6.32) were increased in negative control group when compared to our vehicle control group that had a cholesterol level of 90±1.21mg/dL, triglycerides level of 31±2.12mg/dL and an LDL level of 49.8±1.21mg/dL. Upon treatment with metformin, these levels didn't decrease much. Even the diet reversal could not ameliorate the increased cholesterol levels (182±1.13mg/dL) (Figure 6.27), triglycerides (169±2.08mg/dL) (Figure 6.29), and LDL (76.2mg/dL) (Figure 6.33). But upon treatment with phytochemicals, UMB, and GABA these levels were significantly reduced. UMB when given to the diet reversal group in low doses ameliorated cholesterol (77±0.73mg/dL), triglycerides (26±2.83mg/dL), and LDL (40.8±3.84mg/dL) levels better than the high dose of UMB. GABA also decreased cholesterol (103±1.186mg/dL), triglyceride (51±3.573mg/dL), and LDL (41.3±2.85mg/dL) levels in a dose-dependent manner as its low dose was better in ameliorating these levels. Plenty of reports have supported the fact that LDL and VLDL lead to obesity and heart-related diseases in T2DM patients (Kobayashi et al., 2001; Noeman et al., 2011) which was also evident in our study.

HDL often referred to as good cholesterol, is needed for the healthy functioning of the body (Toth, 2005). The HDL levels were found to be decreased in the negative control group (29±2.78mg/dL) (Figure 6.30).

When metformin was given to the positive control group (34±0.45mg/dL), it did not ameliorate it. And even diet reversal alone could not help ameliorating the decreased HDL levels as HDL level was 31±1.2mg/dL in the DR-negative control group. But upon treatment with the phytochemicals along with DR, the decreased HDL level increased when compared to the vehicle control group. UMB low dose in the diet reversal group brought HDL level to 64±2.1 mg/dL while GABA low dose in the diet reversal group brought HDL levels to 69±0.31mg/dL (Figure 6.31).

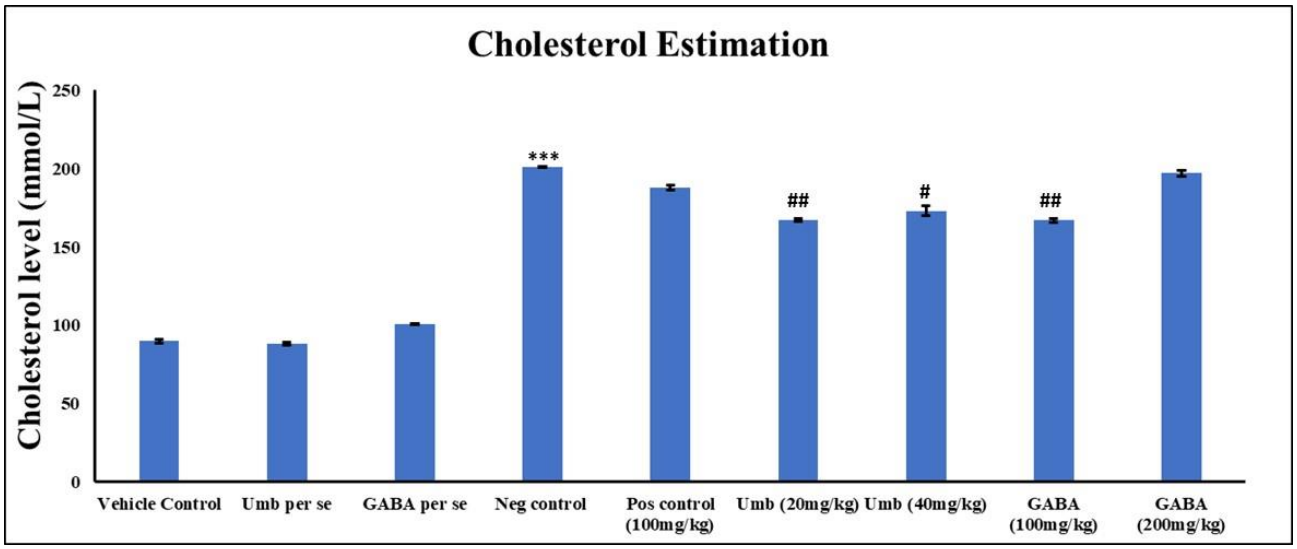


Figure 6.26: Effect of GABA and UMB treatment on Cholesterol levels in the HFD group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05 vs negative control

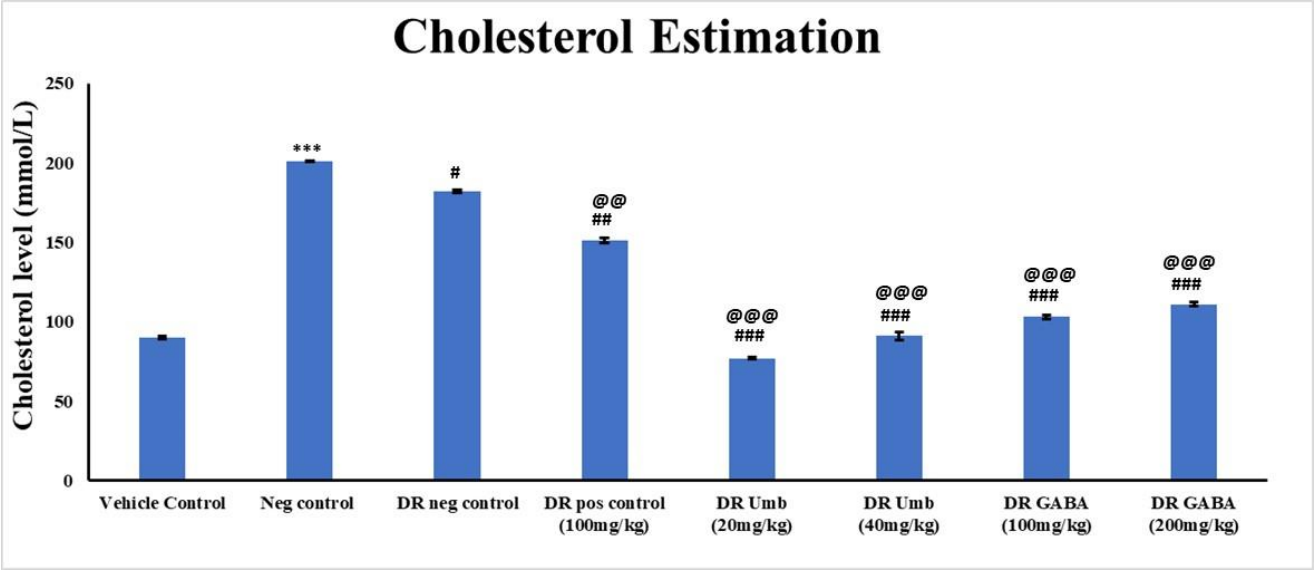


Figure 6.27: Effect of GABA and UMB treatment on cholesterol levels in the DR group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

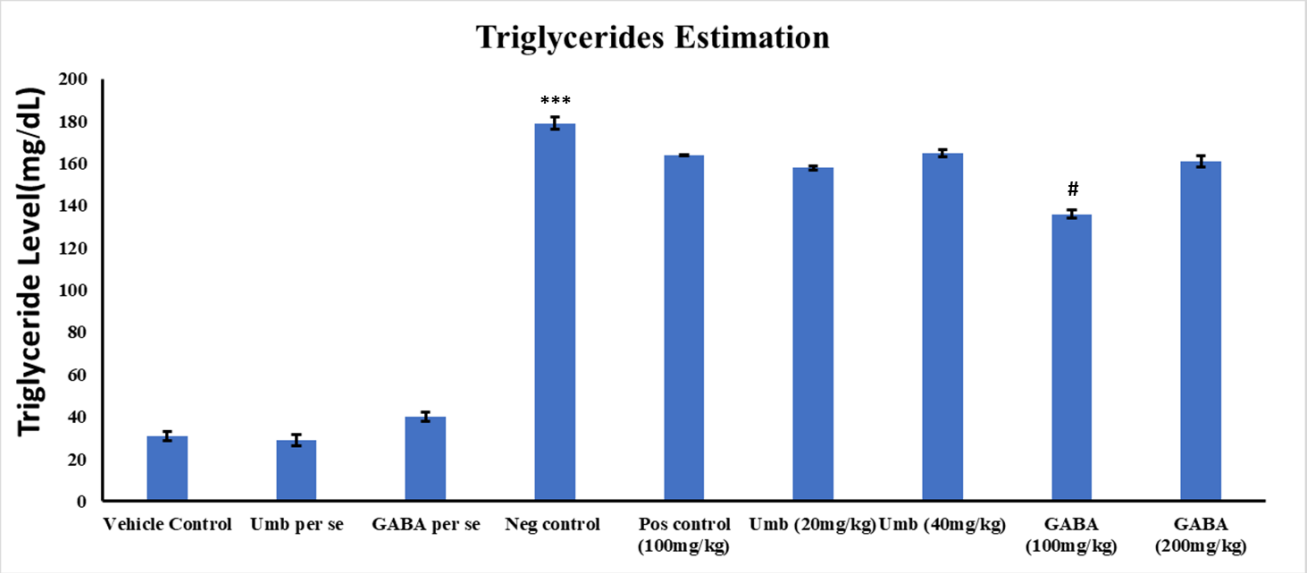


Figure 6.28: Effect of GABA and UMB treatment on triglyceride levels in the HFD group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05 vs negative control

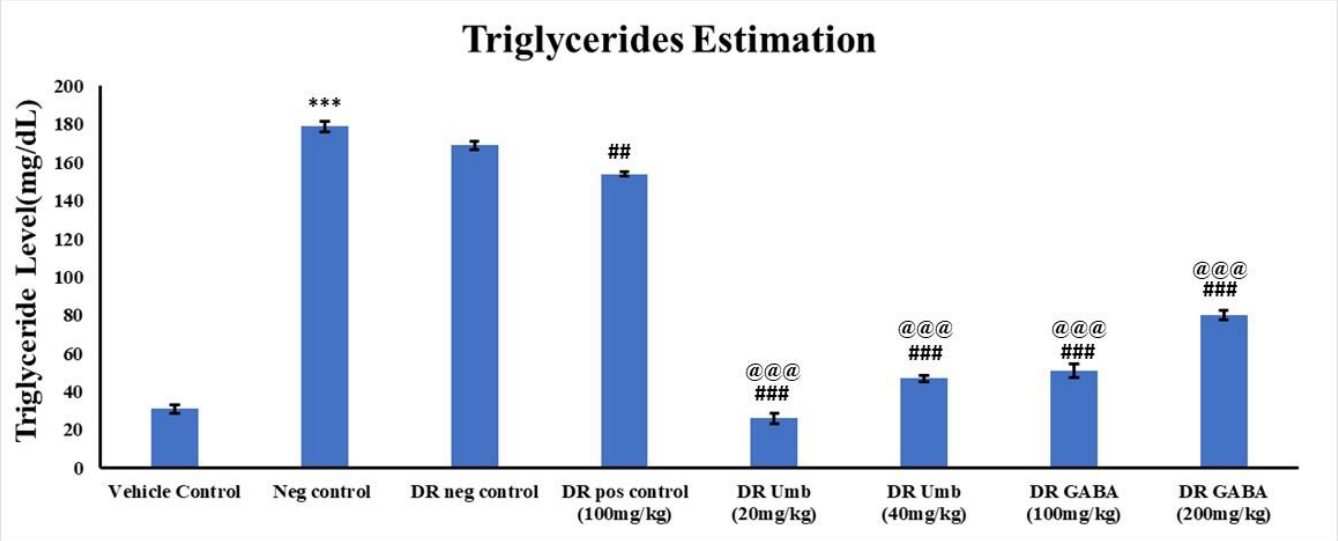


Figure 6.29: Effect of GABA and UMB treatment on triglyceride levels in the DR group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

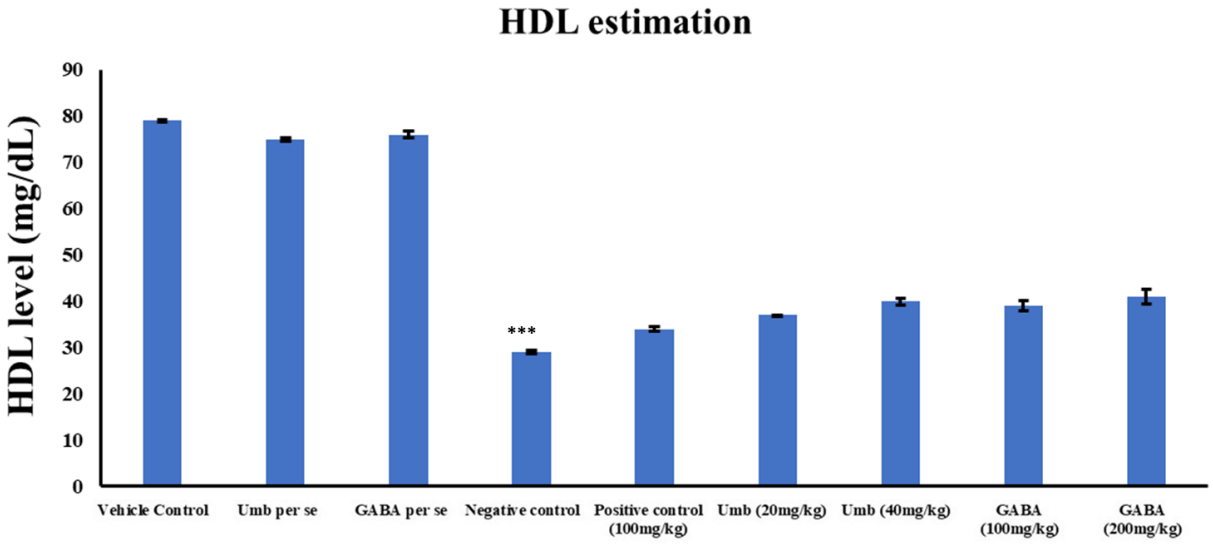


Figure 6.30: Effect of GABA and UMB treatment on HDL level in the HFD group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05 vs negative control

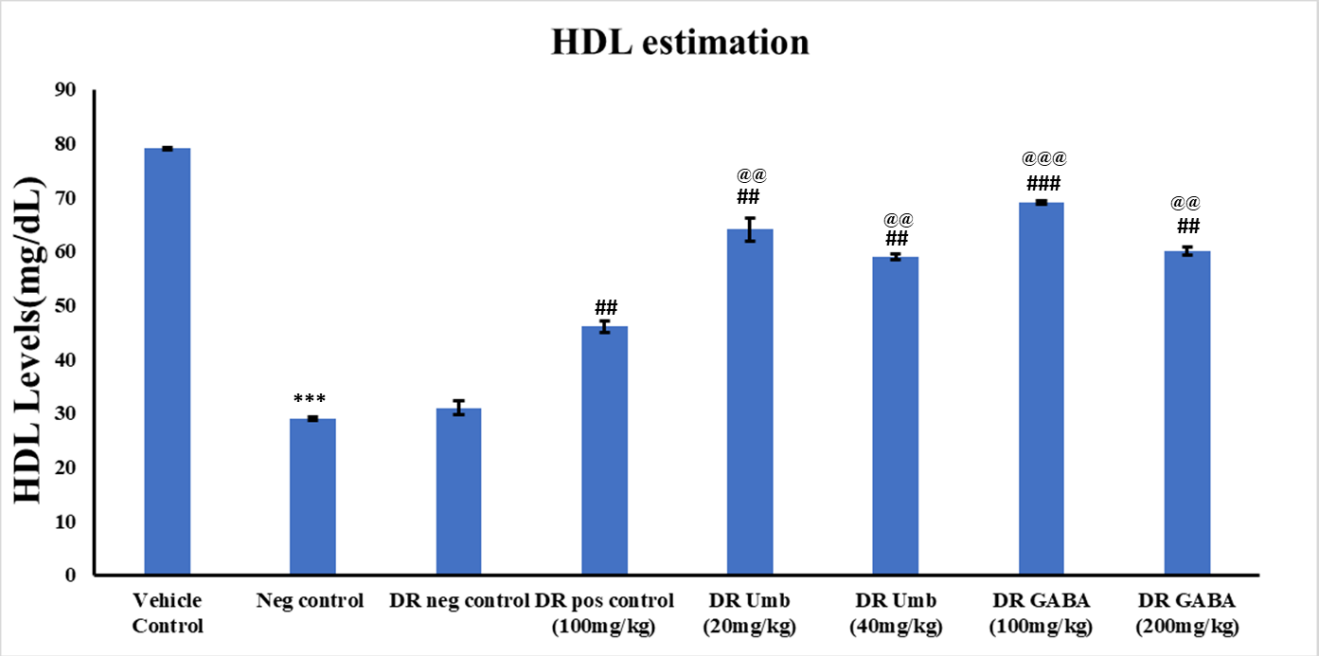


Figure 6.31: Effect of GABA and UMB treatment on HDL level in the DR group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

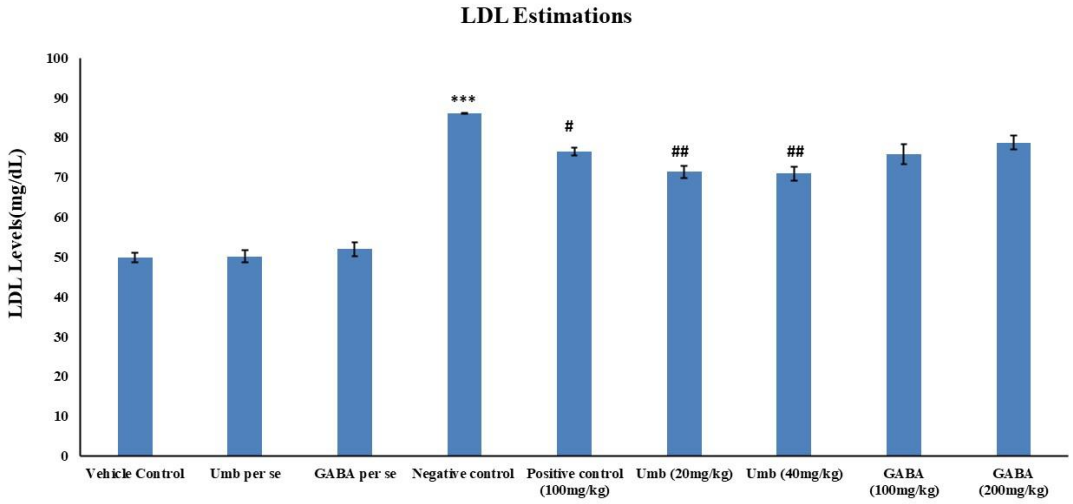


Figure 6.32: Effect of GABA and UMB treatment on LDL level in the HFD group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05 vs negative control

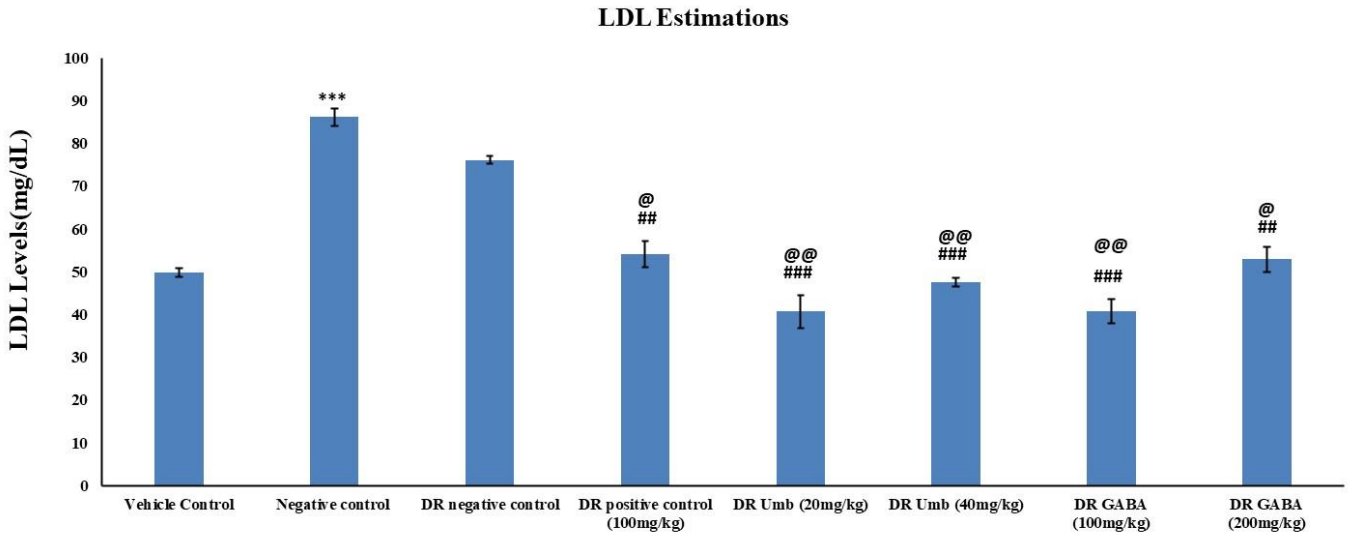


Figure 6.33: Effect of GABA and UMB treatment on LDL level in the DR group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.7.3.2 Renal Function Test

Three parameters BUN, creatinine, and uric acid levels were checked to assess how well the kidney is functioning. BUN (73±1.86mg/dL) (Figure 6.34), creatinine (0.62±0.014mg/dL) (Figure 6.36), and uric acid levels (1.6±002mg/dL) (Figure 6.38) were found to be increased in the negative control group when compared to the vehicle control group that had BUN (39±2.14mg/dL), creatinine (0.42±0.01mg/dL) and uric acid (0.5±0.03mg/dL). Upon treatment with metformin, the positive control group had BUN 64±1.99mg/dL, creatinine 0.54±0.01mg/dL, and uric acid 1.3±0.016mg/dL suggesting no change in these levels. DR also could not bring down the increased BUN level as DR negative control group had BUN 69±2.76mg/dL (Figure 6.35), creatinine (0.43±0.025mg/dL) (Figure 6.37), and uric acid (1.6±0.016mg/dL) (Figure 6.39). But when phytochemical was given to the DR groups it ameliorated renal functions in a dose-dependent manner. UMB low dose brought BUN, creatinine, and uric acid levels to 51±1.16mg/dL, 0.41±0.0096mg/dL, and 1±0.0131 mg/dL respectively while GABA low dose brought BUN, creatinine, and uric acid levels to 48±2.83mg/dL, 0.41±0.015mg/dL and 0.4±0.014mg/dL respectively.

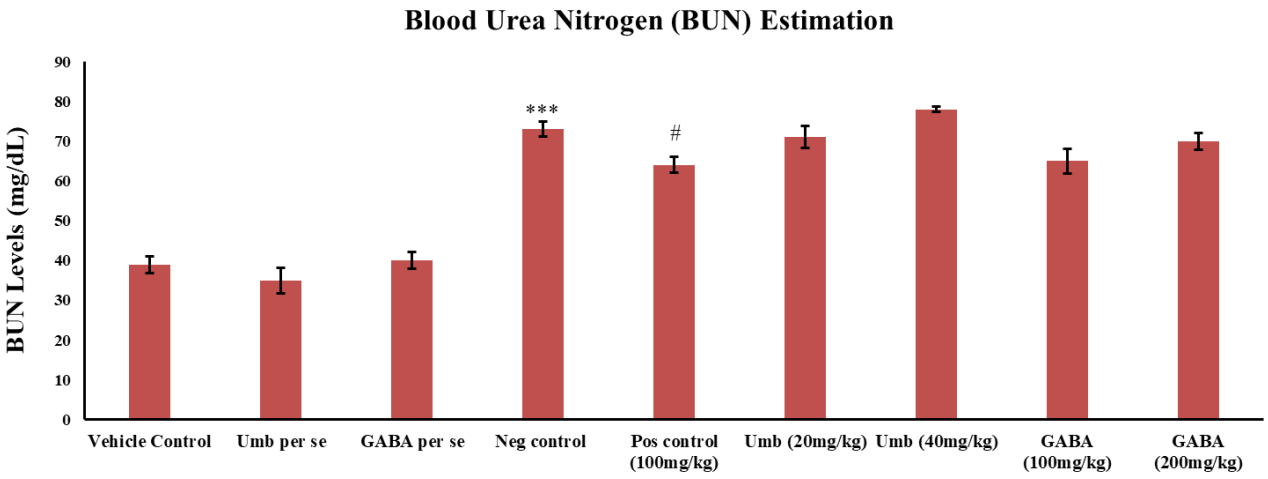


Figure 6.34: Effect of GABA and UMB treatment on BUN level in the HFD group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05 vs negative control

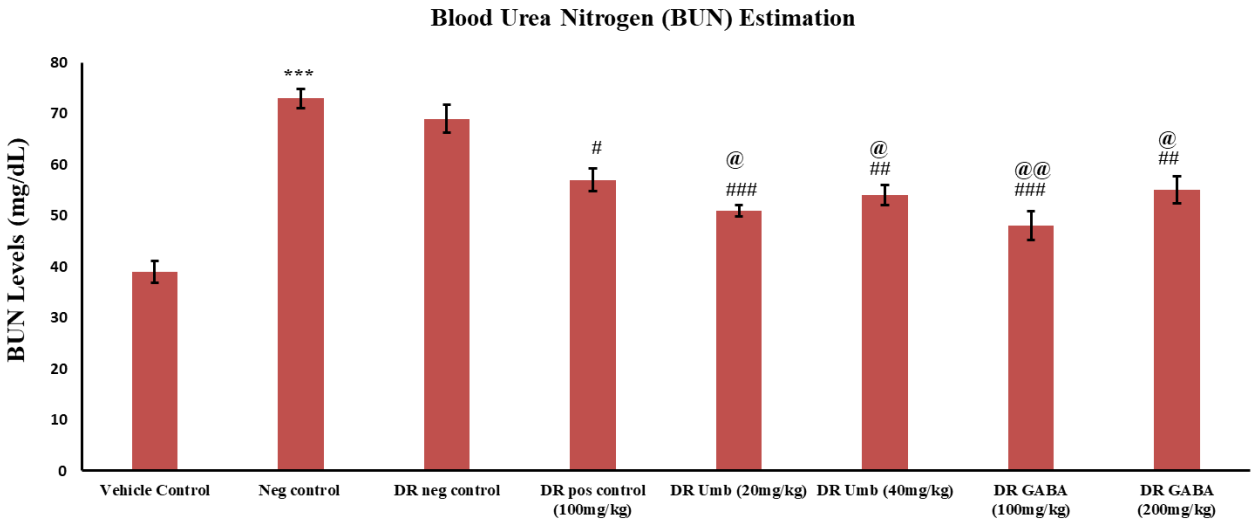


Figure 6.35: Effect of GABA and UMB treatment on BUN level in the DR group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

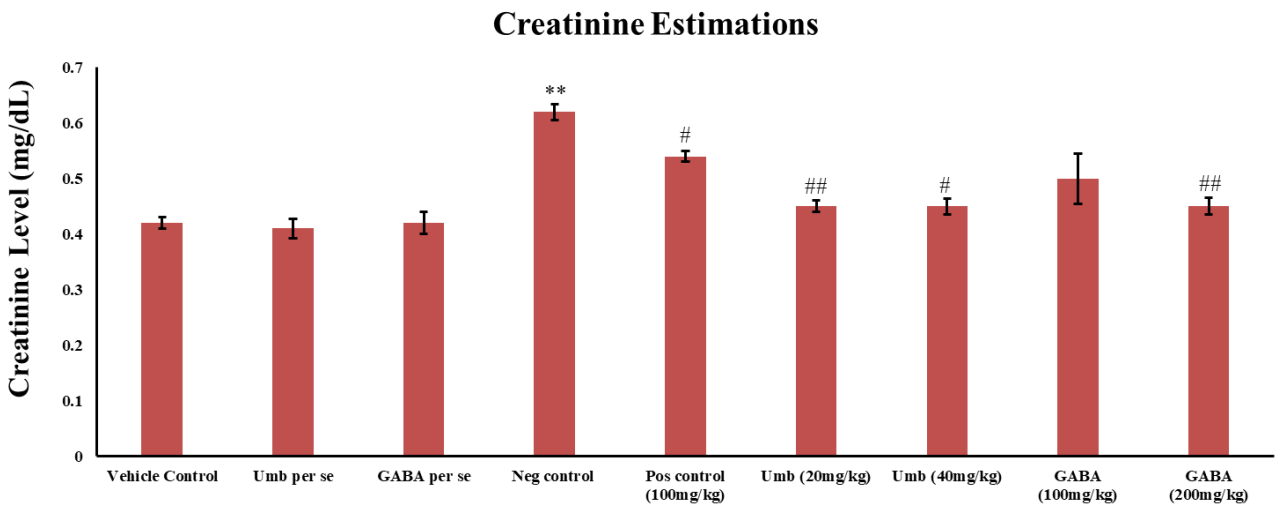


Figure 6.36: Effect of GABA and UMB treatment on Creatinine level in HFD group after the study.

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05 vs negative control

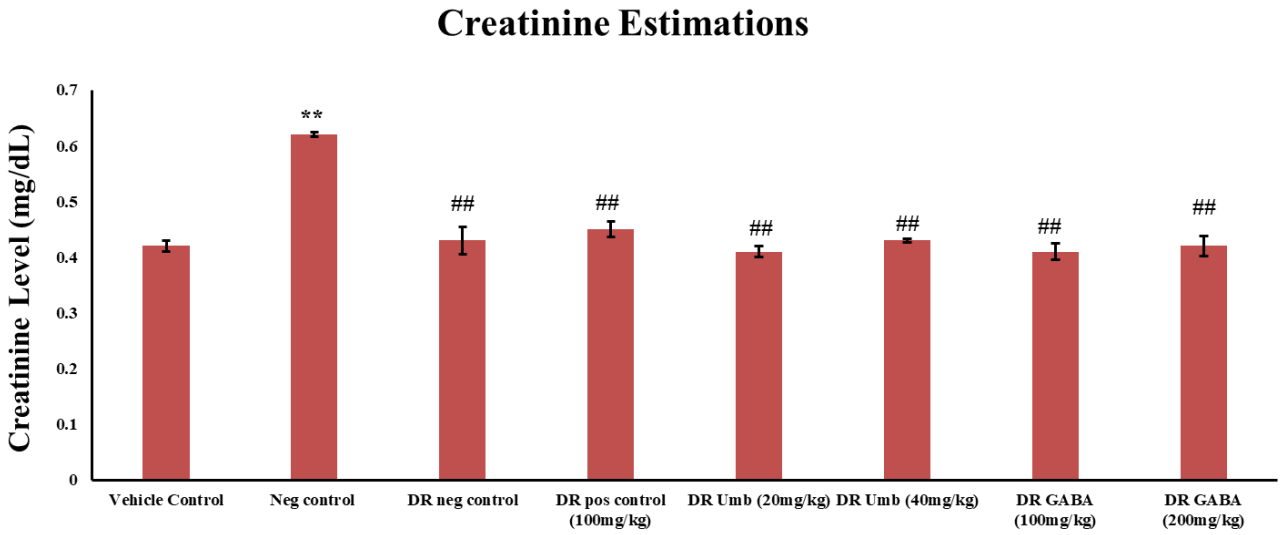


Figure 6.37: Effect of GABA and UMB treatment on Creatinine level in DR group after the study

All the values are represented as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

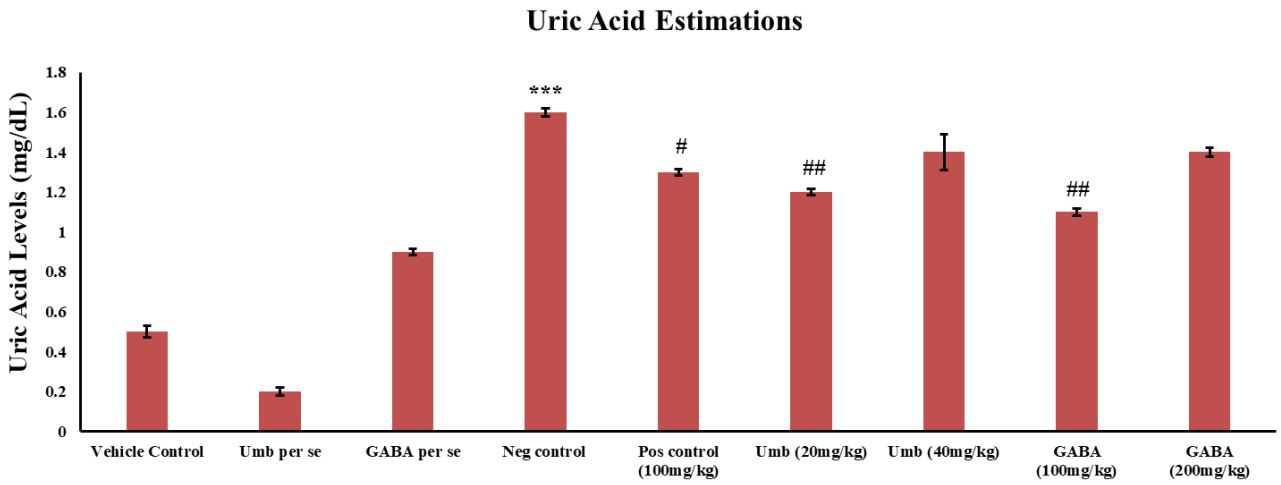


Figure 6.38: Effect of GABA and UMB treatment on Uric Acid level in HFD group after the study.

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

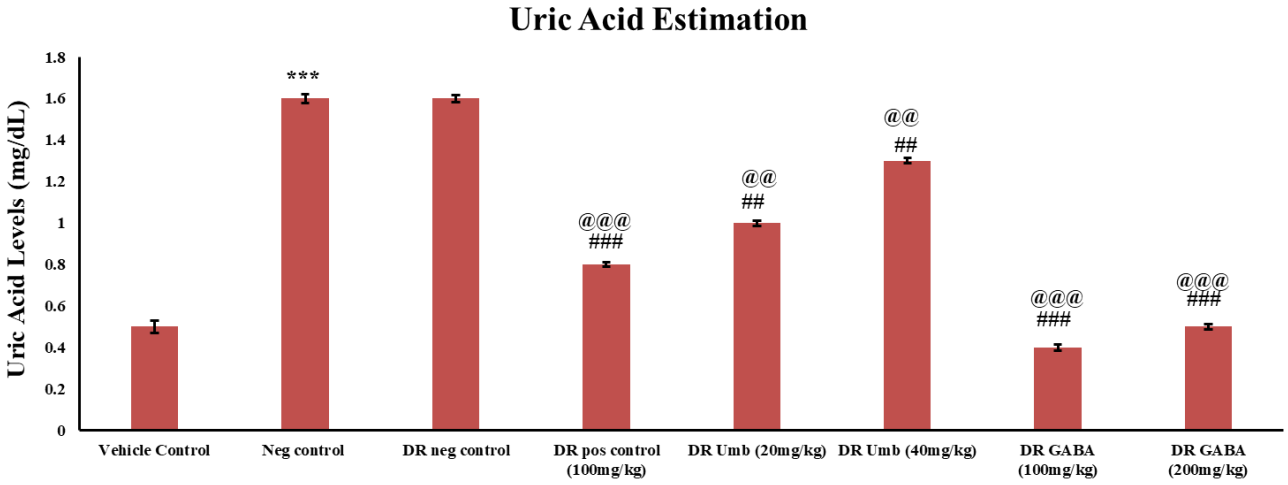


Figure 6.39: Effect of GABA and UMB treatment on Uric Acid level in DR group after the study

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.7.3.3 Liver Function Test

Four parameters Bilirubin, SGOT/ aspartate aminotransferase (AST), SGPT/ alanine transaminase (ALT), and ALP were investigated in liver profile to assess liver was functioning and up to what extent HFD can affect its functioning. Bilirubin (0.35mg/dL) (Figure 6.40), SGOT (195±3.96U/L) (Figure 6.42), SGPT (132±2.41U/L) (Figure 6.44), and ALP (851±9.12U/L) levels (Figure 6.46) were found to be elevated in the negative control group when compared with the Vehicle control group that had bilirubin (0.06mg/dL), SGOT (82±4.32U/L), SGPT (75±1.12U/L) and ALP (309±15.21U/L). Treatment with metformin did not ameliorate the increased levels of bilirubin (0.32mg/dL), SGOT (163±4.85U/L), SGPT (126±5.74U/L), and ALP (651±10.84U/L) in the positive control group. DR alone could not decrease the Bilirubin (0.28±0.01mg/dL) (Figure 6.41), SGOT (177±5.96U/L) (Figure 6.43), SGPT (124±2.41U/L) (Figure 6.45) and ALP (756±9.35U/L) levels (Figure 6.47) in the DR negative control group but when phytochemicals were given to the DR group, it significantly ameliorated these levels. UMB low dose brought bilirubin, SGOT, SGPT, and ALP levels to 0.14mg/dL, 82±7.12U/L, 73±1.541U/L, and 253±2.45U/L respectively while GABA low dose brought bilirubin, SGOT, SGPT, ALP level to 0.13±0.01mg/dL, 81±2.88U/L, 75±5.54U/L, and 265±16.21U/L respectively.

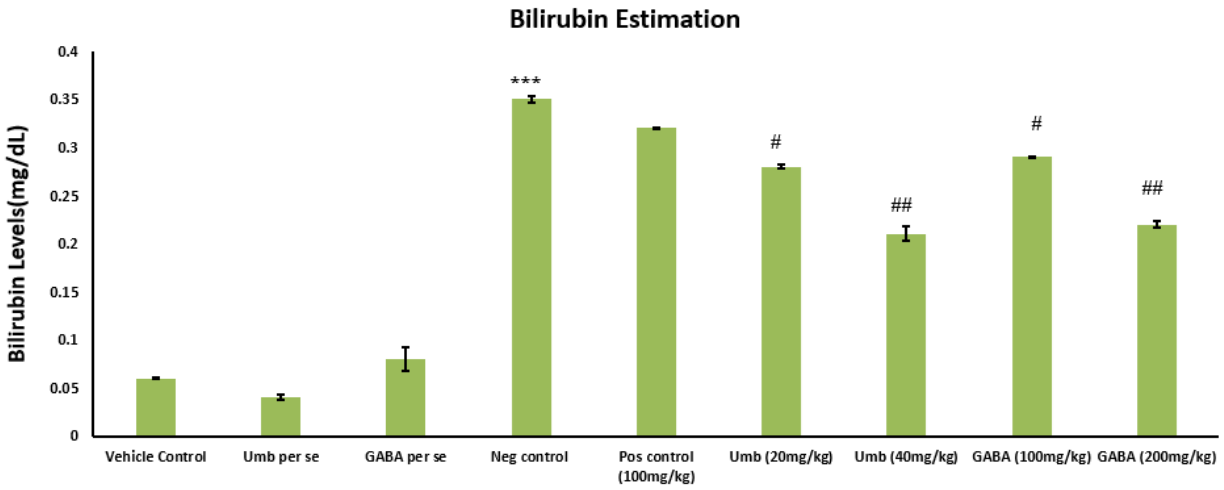


Figure 6.40: Effect of GABA and UMB treatment on Bilirubin level in the HFD group after the study.

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

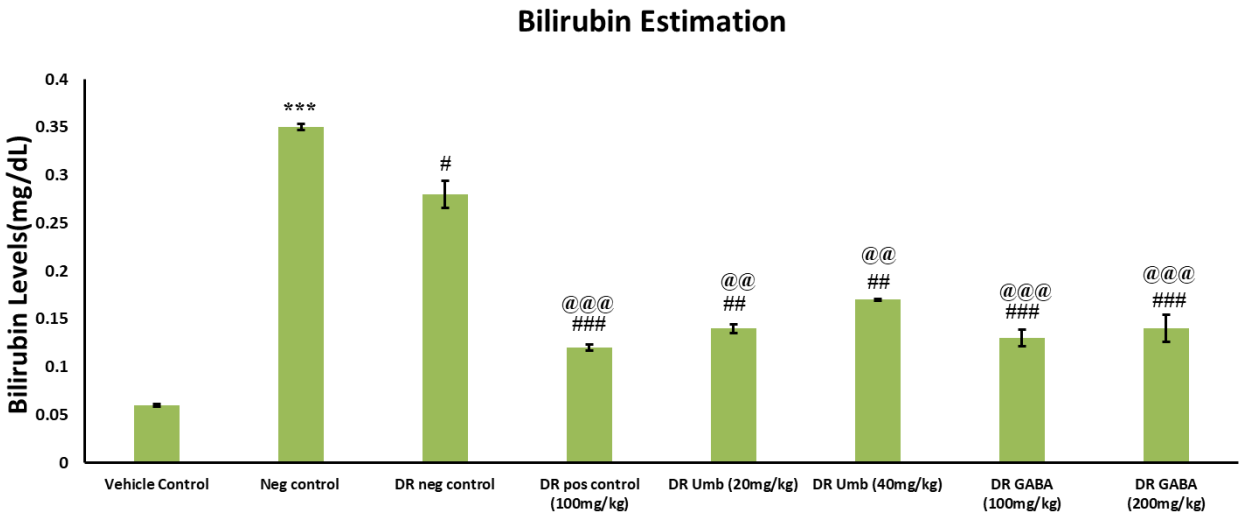


Figure 6.41: Effect of GABA and UMB treatment on Bilirubin level in the DR group after the study

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

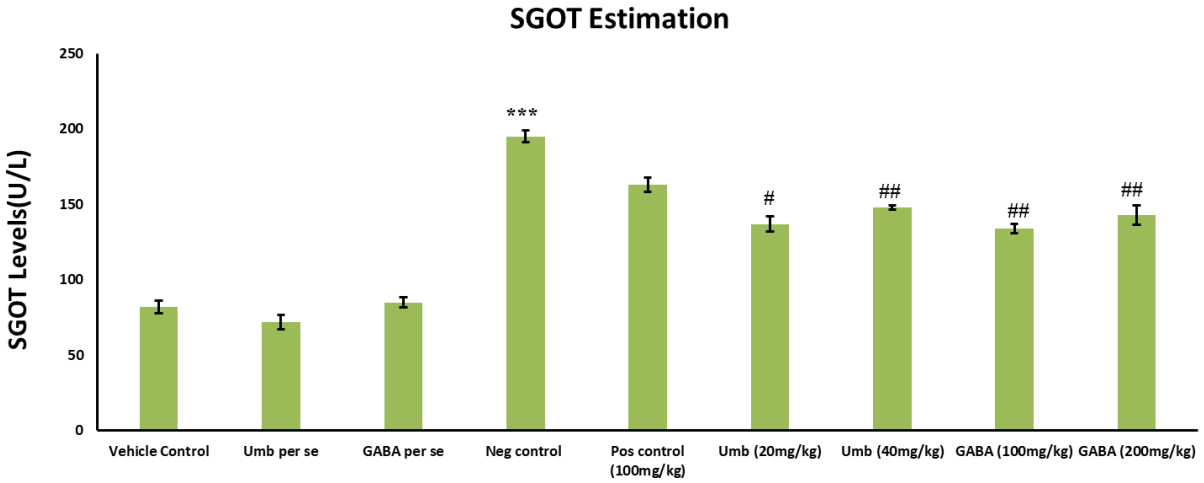


Figure 6.42: Effect of GABA and UMB treatment on SGOT level in the HFD group after the study

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

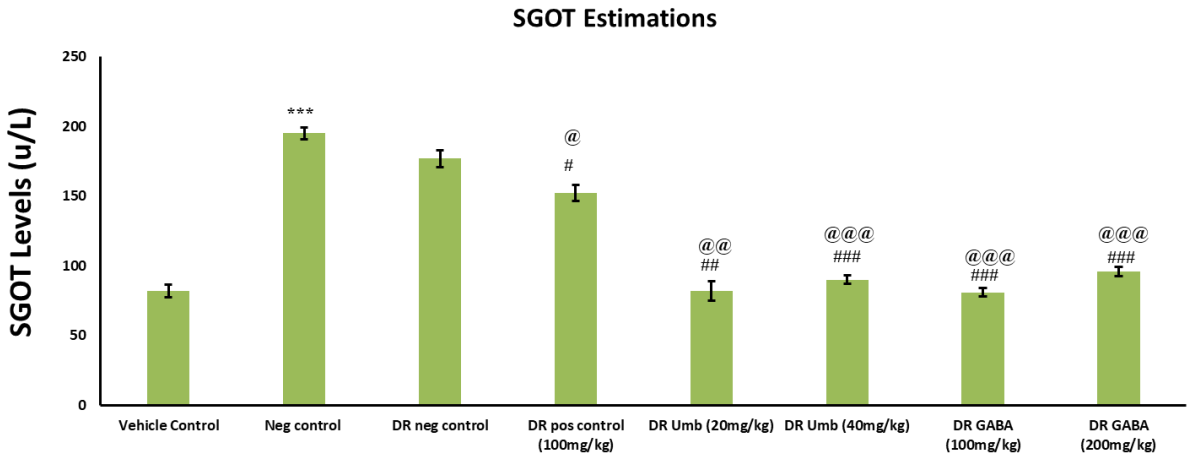


Figure 6.43: Effect of GABA and UMB treatment on SGOT level in the DR group after the study

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

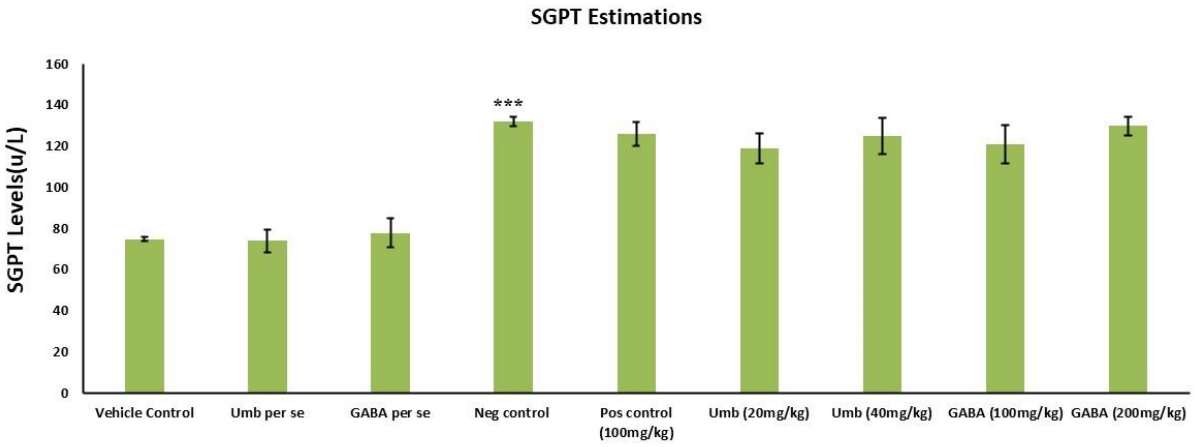


Figure 6.44: Effect of GABA and UMB treatment on SGPT level in the HFD group after the study

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control.

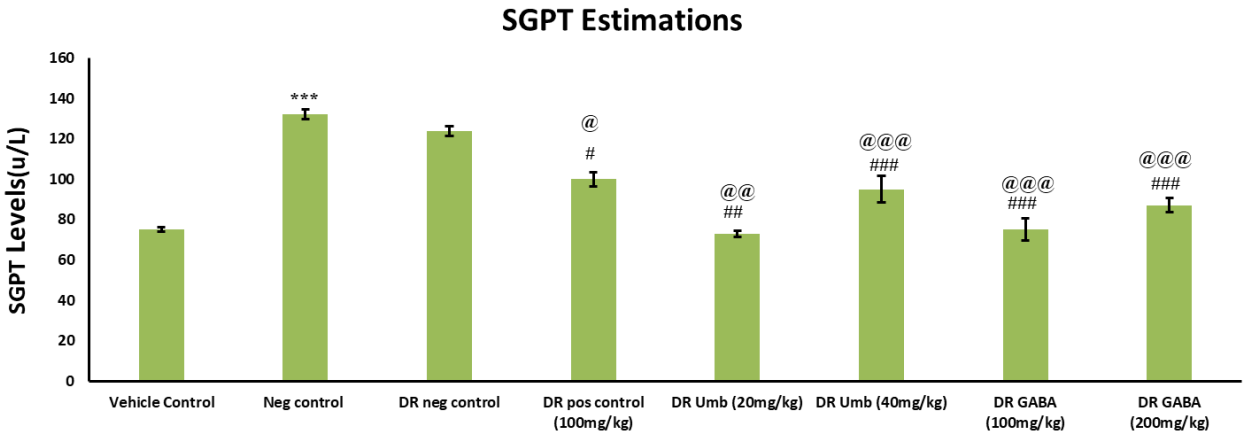


Figure 6.45: Effect of GABA and UMB treatment on SGPT level in the DR group after the study

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

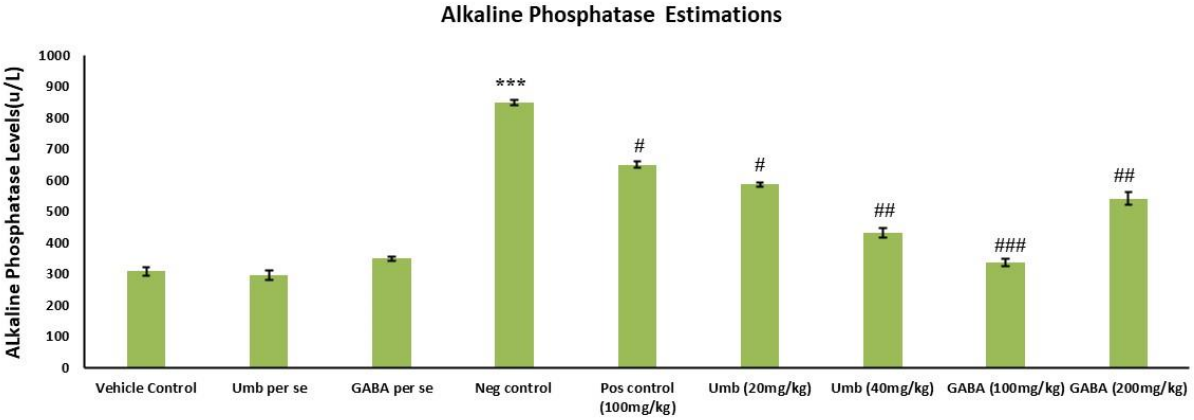


Figure 6.46: Effect of GABA and UMB treatment on ALP level in the HFD group after the study

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

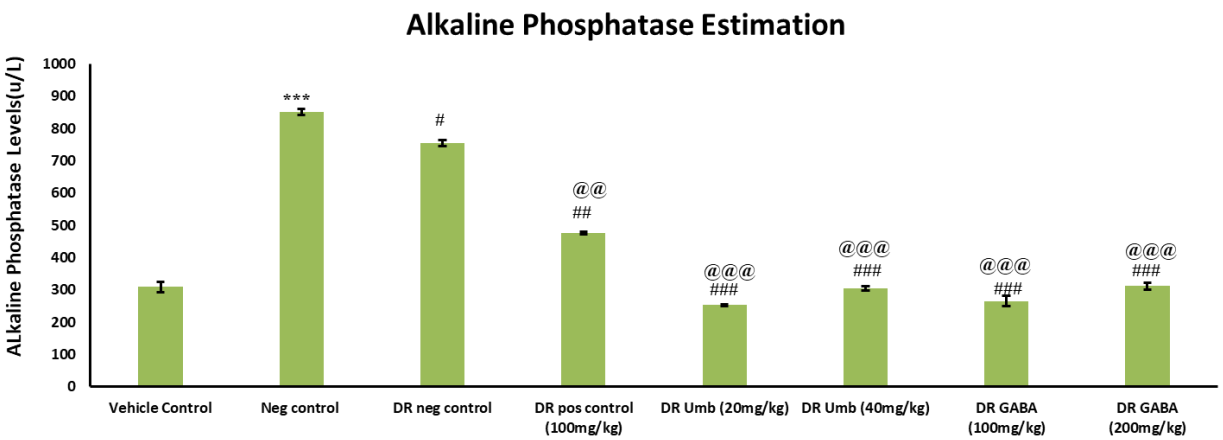


Figure 6.47: Effect of GABA and UMB treatment on ALP level in the DR group after the study

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.7.4 Histopathology

Histopathology was done on the kidney of the rats. The tissue was stained by Hematoxylin and Eosin dye followed by viewing at 40x magnification.

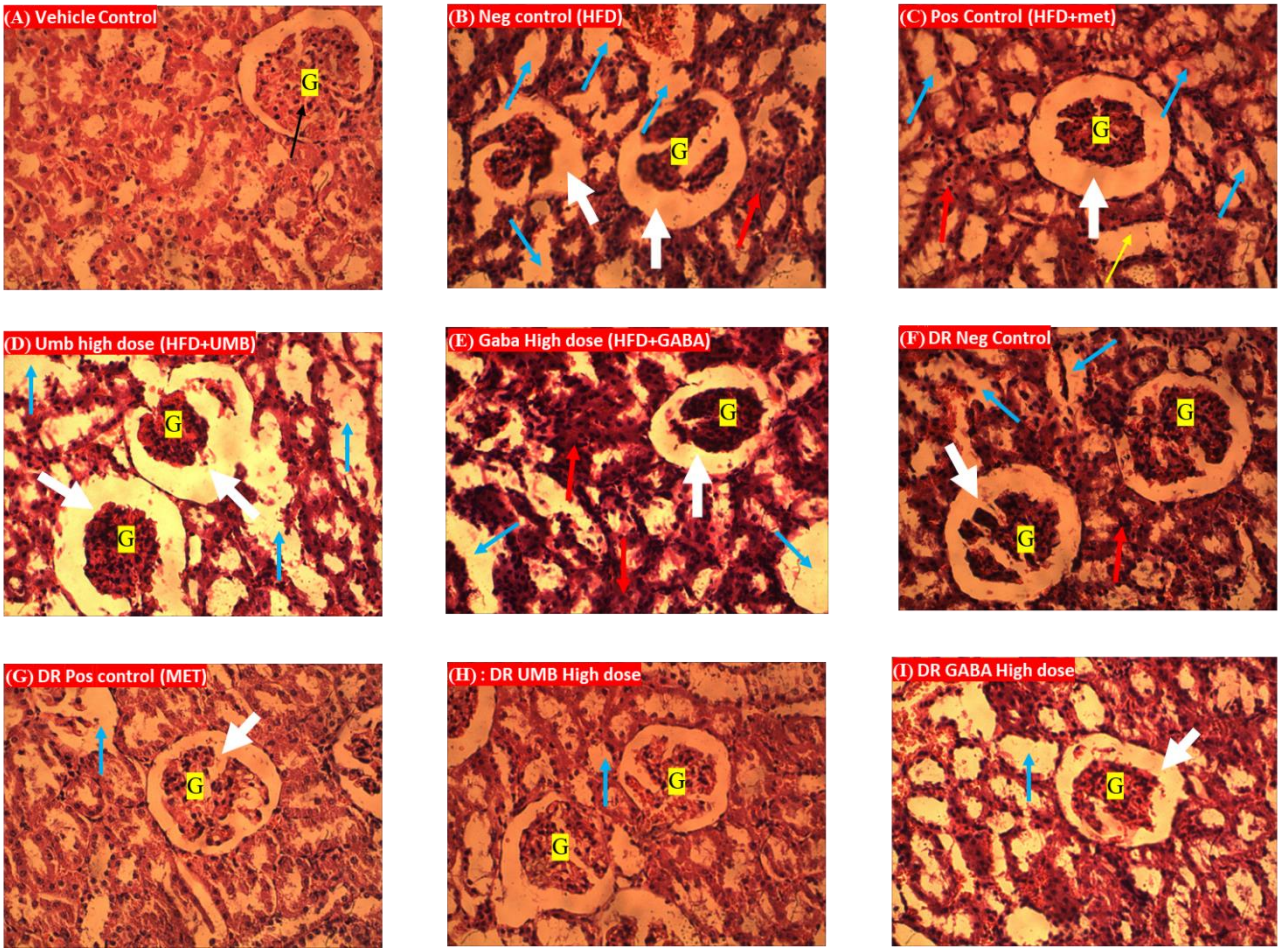


Figure 6.48: Histopathology of the kidney at the end of the study

Legends:

Pointing Normal Glomeruli G **Tubular atrophy**

Disturbed Bowman's Capsule and Glomerular tuft area

Inflammation is seen as cells clustered together

Histopathology of the kidney revealed the extent of glomerulosclerosis. We can see glomerulus is distorted in the negative control group and treatment with metformin in the positive control group did not affect much (Figure 6.48). It also shows marked glomerular atrophies with diffused mesangial distribution throughout the cortex as compared to the vehicle control group. We can see in (G), (H), and (I) that treatment of respective phytochemicals along with DR shows moderate recovery and normal appearance of glomerulus with less inflammation when compared to negative control and DR negative control.

6.8 In vivo oxidative marker

6.8.1 Protein estimation in tissue homogenate using Lowry's method

To express the specific activity of enzymes, the total protein content is employed. Using the established technique outlined by Lowry et al., (Figure 6.49).

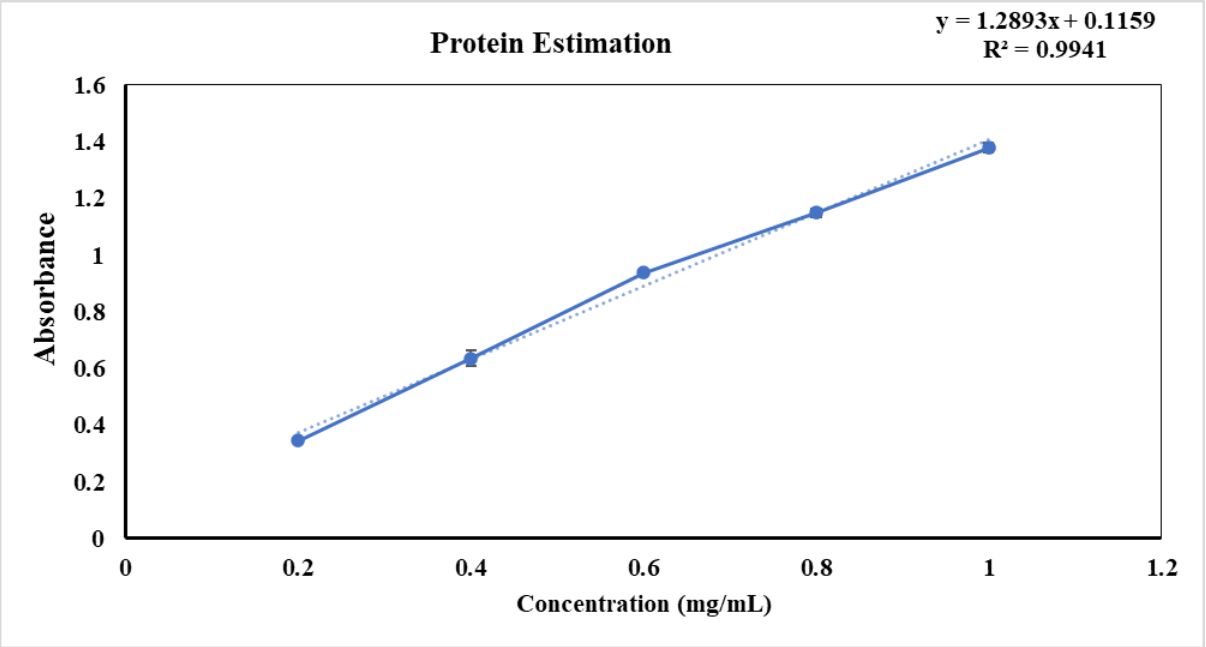


Figure 6.49: Total protein Estimation using lowry assay

6.8.2 Oxidative Biomarkers:

6.8.2.1 LPO:

During the diabetic condition, the high blood glucose and high lipid content in adipose tissue, result in the increased size of adipocytes, which in turn results in the synthesis of phospholipase A2. LPO results from phospholipase A2 activation (Spiteller et al.,2003). TBARS assay was used to determine the MDA levels described by (Tsikas, 2017). No substantial change was seen in MDA levels of the vehicle control group (0.07±0.015µMol MDA/mg), while MDA levels were elevated in the negative control group (1.64±0.07015µMol MDA/mg) (Figure 6.50) upon treatment with the positive control (metformin) it didn't change MDA levels much (1.56±0.06µMol MDA/mg). Treatment with phytochemicals in the HFD treated group didn't ameliorate the increased MDA levels. DR alone didn't bring MDA levels down as DR negative control group had MDA levels of (1.16±0.02 µMol MDA/mg). But when phytochemical was administered to the DR group it brought MDA levels down in a dose-dependent manner. UMB low dose in the DR group brought MDA levels to 0.4±0.013 µMol MDA/mg) while UMB high dose in the DR group brought MDA levels to 0.55±0.02 µMol MDA/mg. GABA low dose in the DR group brought MDA levels to 0.59±0.008 µMol MDA/mg) while GABA high dose in the DR group brought MDA levels to 0.74±0.005 µMol MDA/mg (Figure 6.51).

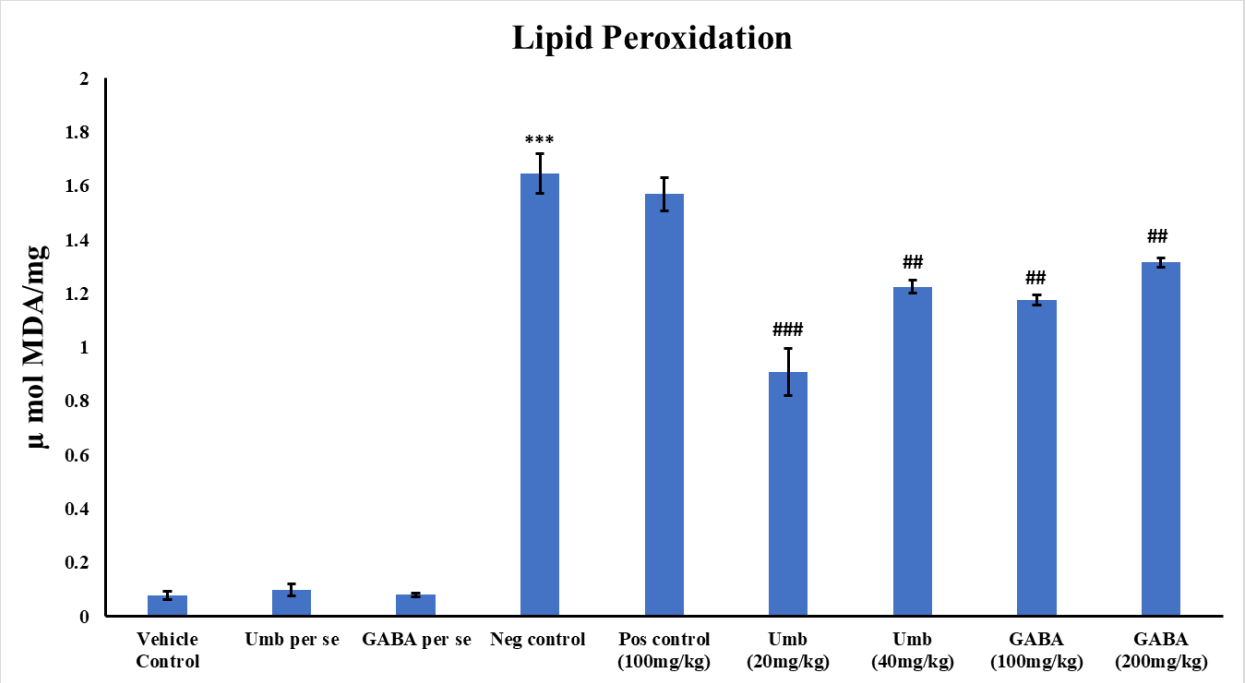


Figure 6.50: Effect of GABA and UMB treatment on LPOin post-nuclear supernatant of HFD group.

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

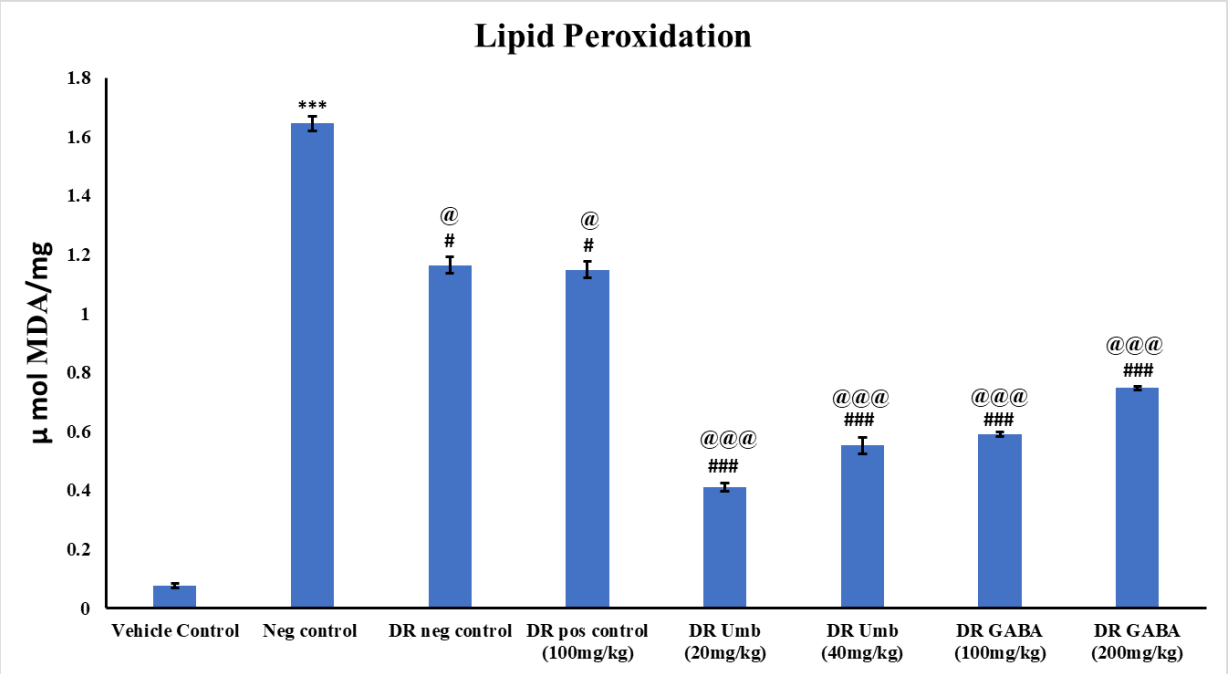


Figure 6.51: Effect of GABA and UMB treatment on LPO in post-nuclear supernatant of tissue of DR

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.8.2.2 Catalase

All living things exposed to oxygen include CAT, a crucial enzyme that protects cells from oxidative stress. It works by breaking down hydrogen peroxide into water and oxygen, which neutralizes ROS. The Aebi et al., 1998 approach was used to measure the CAT activity. Catalase levels were decreased in the negative control group (2.07±0.12 U/mg) when compared to the vehicle control group (8.44±0.25 U/mg). Treatment with metformin in the positive control group did not bring the catalyst level down (2.36±0.12 U/mg) (Figure 6.52). Treatment with phytochemicals also did not ameliorate much to the decreased catalase levels. DR also could not ameliorate the decreased catalase levels (2.78±0.14 U/mg). But when phytochemicals were given to the diet reversal group it ameliorated the decreased catalase levels. UMB's low dose brought the catalase level to 6.84±0.305 units for mg while DR GABA brought the catalase level to 6.62±0.13 units for mg (Figure 6.53).

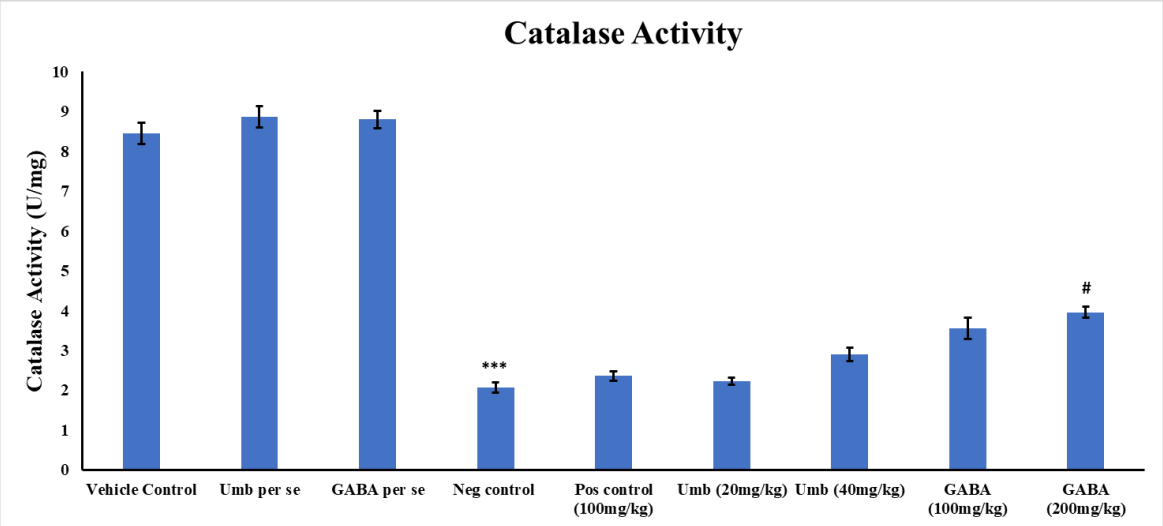


Figure 6.52: Effect of GABA and UMB treatment on Catalase activity in post-nuclear supernatant of tissue of HFD group.

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

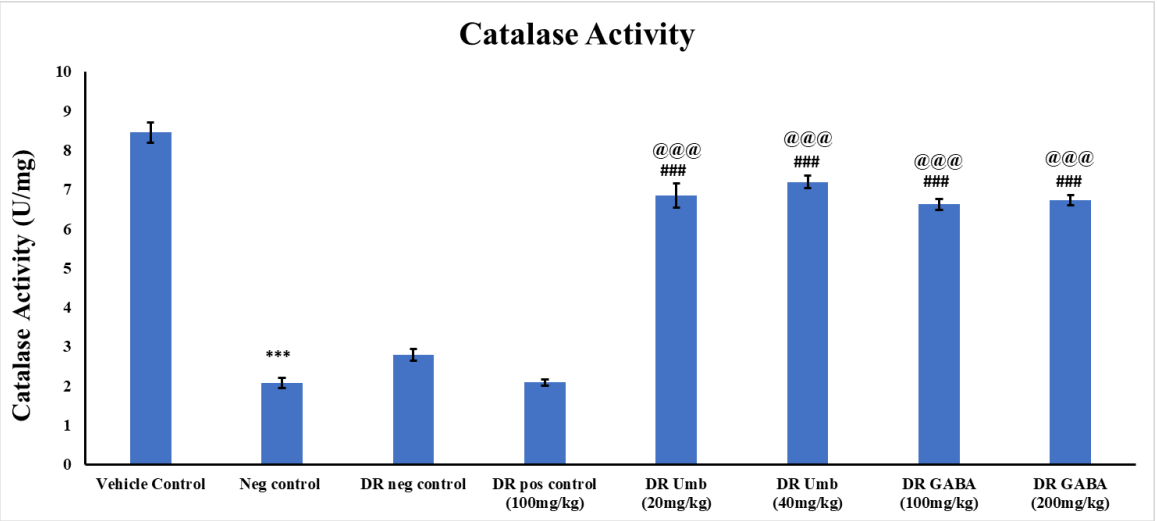


Figure 6.53: Effect of GABA and UMB treatment on catalase activity in post-nuclear supernatant of tissue of DR group.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.8.2.3 SOD

The SOD activity was found to be decreased in the negative control (11.65±2.58 U/mg) when compared to the vehicle control group (115.14±8.23 U/mg) (Figure 6.54). Treatment with metformin and phytochemicals alone couldn't increase SOD levels. Diet reversal increased the SOD level (49.68±1.76 U/mg) but when phytochemicals were administered to the DR it increased SOD levels significantly in a dose-dependent manner. UMB low dose increased SOD activity to 86.09±3.75 U/mg whereas GABA increased SOD activity to 92.45±2.09 U/mg (Figure 6.55).

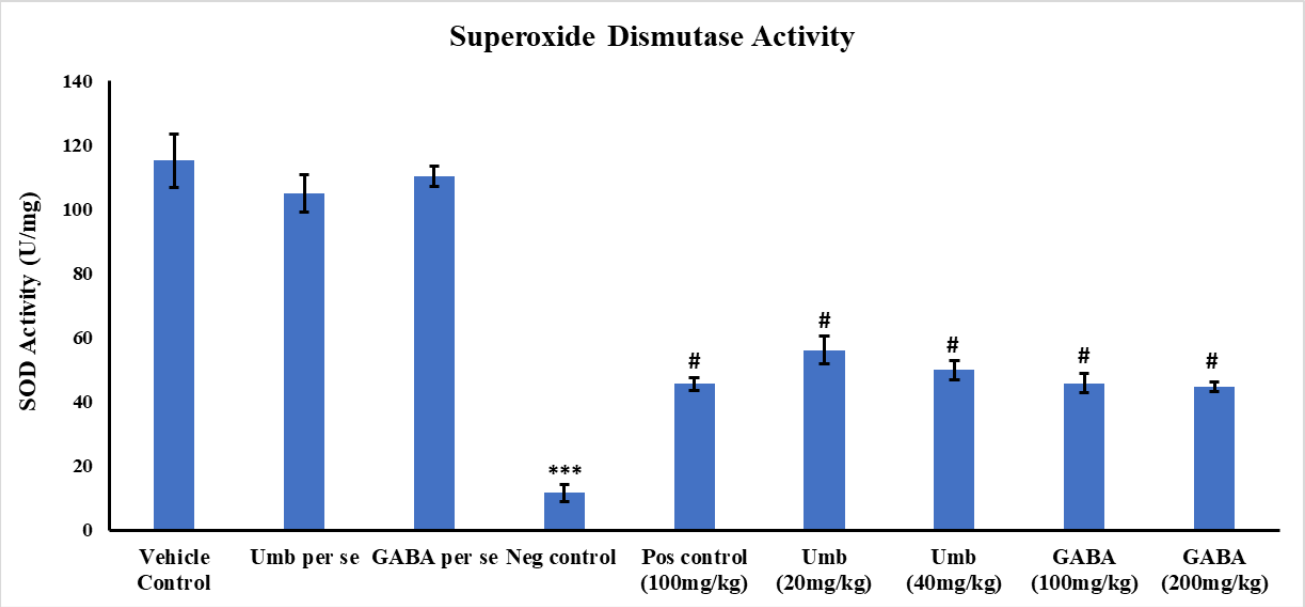


Figure 6.54: Effect of GABA and UMB treatment on SOD activity in post-nuclear supernatant of tissue of HFD group.

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

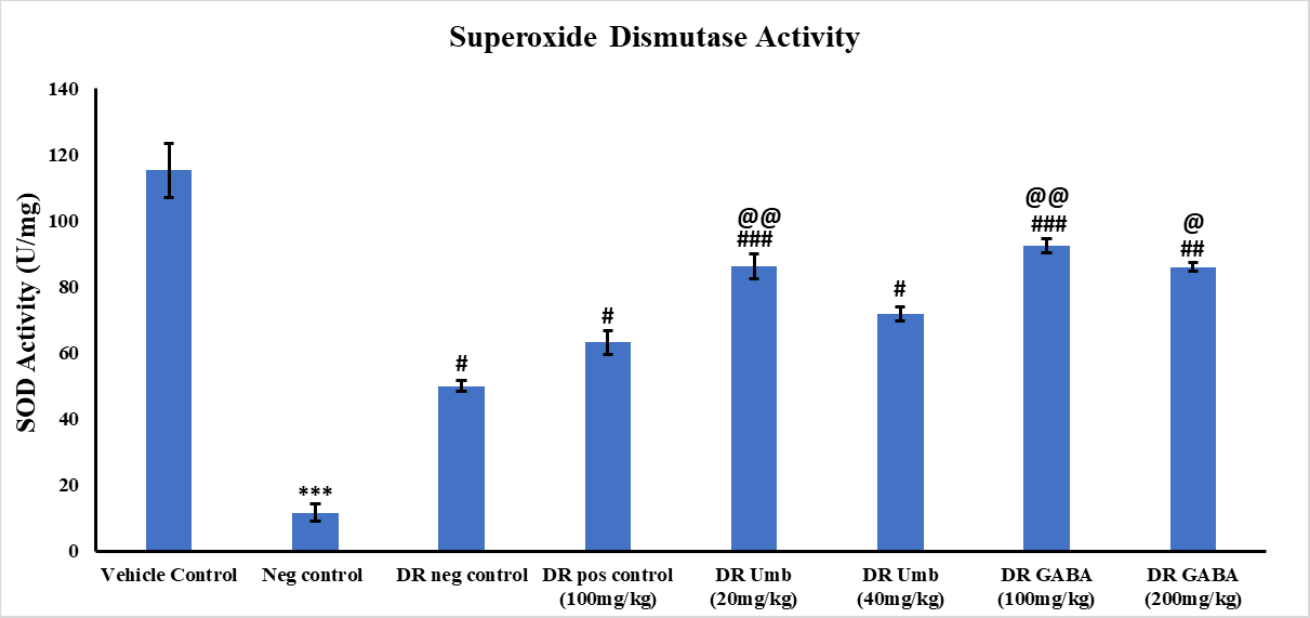


Figure 6.55: Effect of GABA and UMB treatment on SOD activity in post-nuclear supernatant of tissue of DR group.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.8.2.4 GST

GST level was decreased in the negative control (7.23±0.09 U/mg) when compared to vehicle control (49.85±2.03 U/mg) (Figure 6.56). Treatment in the positive control group didn't increase the SOD level (13.96±0.75 U/mg). Diet reversal also couldn't increase the diminished SOD level in the DR neg control (14.36±0.08 U/mg). But when phytochemicals were administered to the DR group it ameliorated the diminished SOD levels in a dose-dependent manner. UMB low dose brought the SOD level to 42.9±0.97 U/mg while GABA low dose brought GST level to 39.26±1.98 U/mg (Figure 6.57).

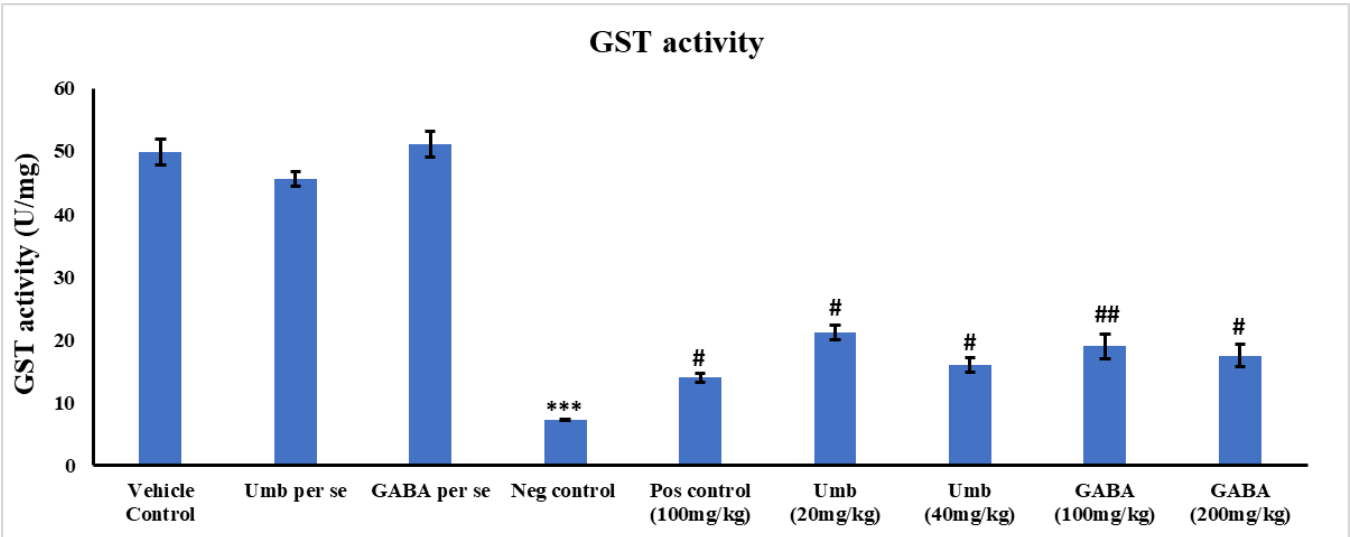


Figure 6.56: Effect of GABA and UMB treatment on GST activity in post-nuclear supernatant of tissue of HFD group.

All the values are shown as mean ± S.E.M. (n=3), ***p<0.001, **p<0.01 and *p<0.05, vs control vehicle control; and ###p<0.001, ##p<0.01 and #p<0.05, vs negative control

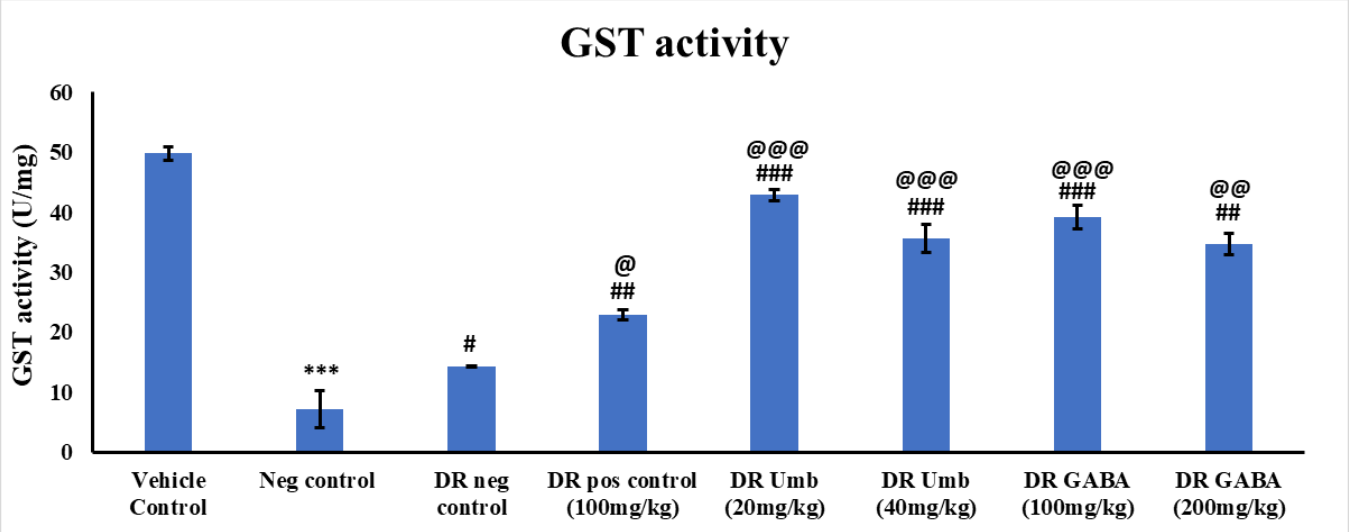


Figure 6.57: Effect of GABA and UMB treatment on GST activity in post-nuclear supernatant of tissue of DR group.

All the values are shown as mean ± S.E.M. (n=3), ###p<0.001, ##p<0.01 and #p<0.05, vs control negative control; ***p<0.001, **p<0.01 and *p<0.05, vs vehicle control; and @@@p<0.001, @@p<0.01 and @p<0.05, vs DR negative control.

6.9 SIRT1 Assay

Tissue homogenate from 8 groups was used to analyse the SIRT1 result. The vehicle control group had a SIRT1 concentration of 7.386±0.015 ng/L while the negative control group had a SIRT1 concentration of 4.392±0.03 ng/L. DR negative control group had a SIRT1 concentration of 3.914±0.01 ng/L while DR positive control group had a SIRT1 concentration of 3.046±0.014 ng/L.

Table 6.27: Concentration of SIRT1 in different animal groups (All the result is expressed as Mean±SEM, where n=3)

Group	Concentration (ng/L)
Vehicle Control	4.386±0.015
Negative Control	4.392±0.03
UMB High Dose	3.865±0.01
GABA High Dose	3.813±0.005
DR negative control	3.914±0.01
DR positive control	3.046±0.014
DR UMB high dose	4.047±0.021
DR GABA high dose	3.258±0.016

6.10 HAc: Change in histone H3 acetylation levels under hyperglycemic conditions in BHK cell line after GABA and UMB treatment.

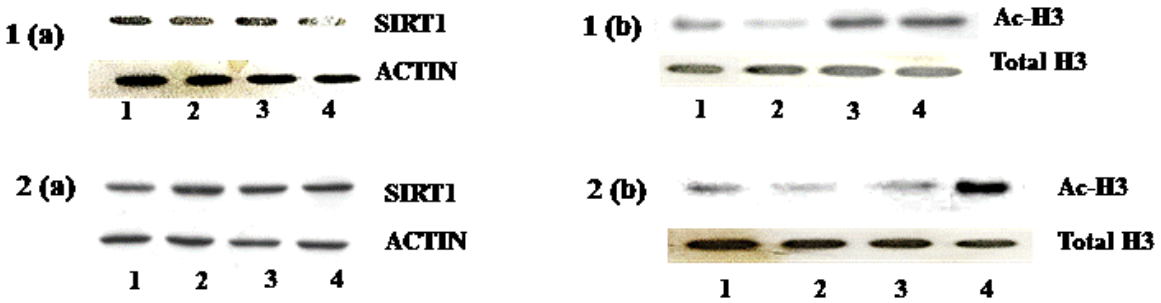


Figure 6.58: Western blot of Acetylation of histone H3 and SIRT1 in 3T3L-1 cell line after treatment with phytochemicals under normal and high glucose conditions. Each lane received 60 µg of protein. To ensure the correct placement of the protein of interest, a pre-stained protein marker (Invitrogen Novex Sharp Pre-Stained Protein Standard, catalogue nUMBer LC5800, Thermo Fisher Scientific) was run alongside the samples.. Actin was employed as an internal control to normalize the results. 1(a) is SIRT1 expression level when UMB was given for the treatment, 1(b) is H3 acetylation when UMB was given as treatment, 2(a) is SIRT1 expression level when GABA was given for the treatment, 2(b) is H3 acetylation when GABA was given as treatment. Lane 1 is low glucose control, Lane 2 is high glucose control, Lane 3 is diet reversal (DR) and Lane 4 is DR+treatment with respective phytochemicals. Values are represented as Mean±SEM from three separate blots.

Our results showed decreased H3Ac under high glucose/hyperglycemic conditions **(Lane 2)** (74.76±2.67) when compared to normal/low glucose conditions (100) **(Lane 1).** Upon reversing the diet, the decreased H3Ac levels increased (142.50±4.96) **(Lane 3)** and upon treatment with GABA along with DR it further increased H3Ac levels (188.00±5.6) **(Lane 4)** and upon treatment with UMB along with DR it further increased H3Ac levels (187.45±6.6) **(Lane 4).**

Our results also demonstrated increased SIRT1 levels in high glucose conditions (111.34±4.5) **(Lane 2)** when compared to low glucose **(Lane 1)** (100). DR decreased the increased SIRT 1 level **(Lane 3)** (98.96±1.73). Upon treatment with GABA along with DR it further decreased SIRT1 levels (94.72±2.25**) (Lane 4)** and upon treatment with UMB along with DR it further decreased SIRT1 levels (84.70±3.45) **(Lane 4).**

Table 6.28: SIRT1 expression level and H3 acetylation level (H3Ac) in 3T3L-1 cell post administration of UMB and GABA.

Lane	Group	H3Ac levels	SIRT1 Levels
1	Low glucose (LG)	100	100
2	High Glucose (HG)	74.76±2.67	93.039±4.5
3	Diet Reversal (DR)	142.50±4.96	92.301±1.73
4	DR+GABA	188.00±5.6	90.776±2.22
1	Low glucose (LG)	100	100
2	High Glucose (HG)	72.05±3.8	95.58±4.76
3	Diet Reversal (DR)	115.67±4.65	102.33±2.88
4	DR+UMB	187.45±6.6	98.65±3.45

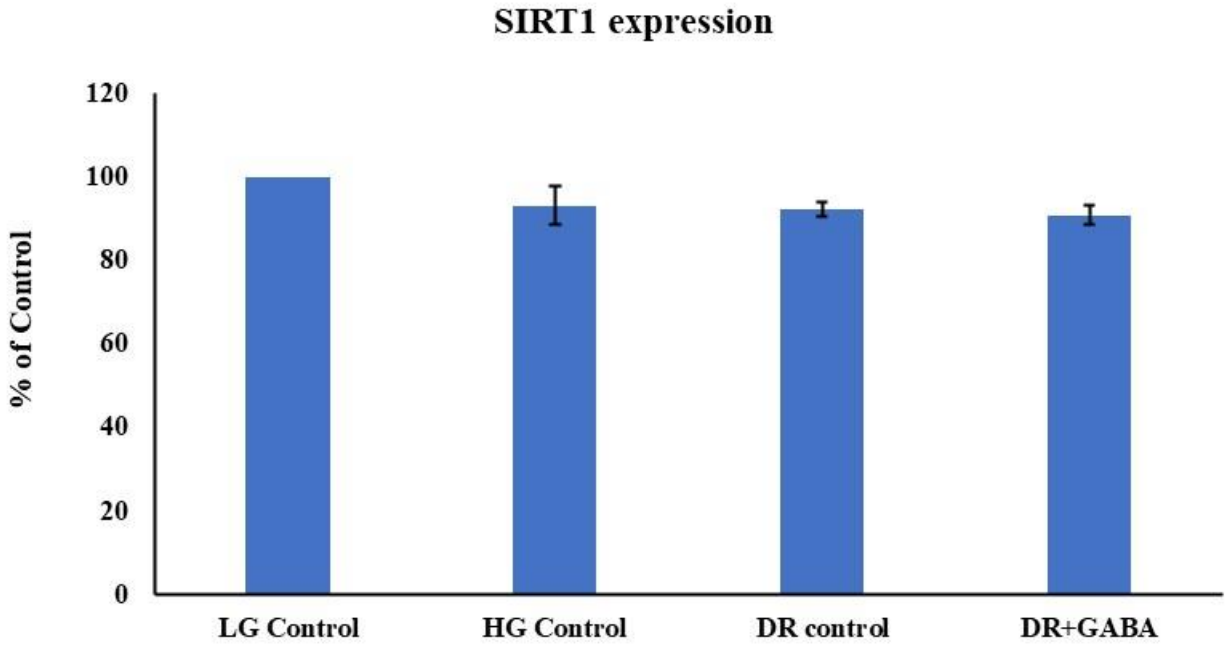
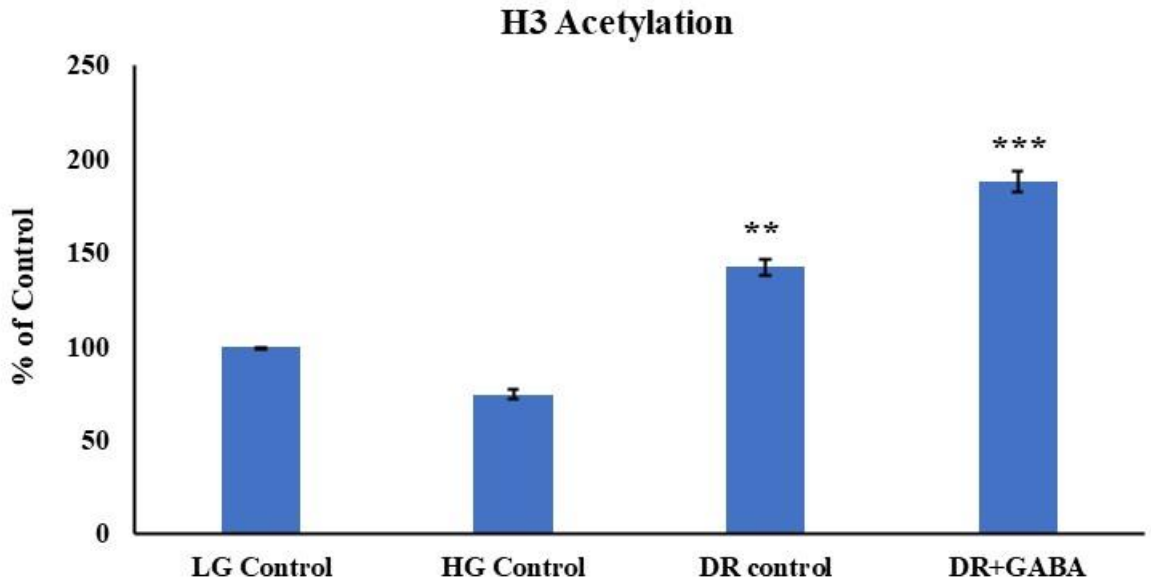


Figure 6.59: SIRT1 expression levels as determined by western blot in 3T3L-1 cell line upon treatment with GABA.



All the values are shown as mean ± S.E.M (with 3 separate blots)

Figure 6.60: Histone 3 acetylation levels as determined by western blot in 3T3L-1 cell line upon treatment with GABA.

All the values are shown as mean ± S.E.M (with 3 separate blots), ***p<0.001, **p<0.01, and *p<0.05, vs control HG

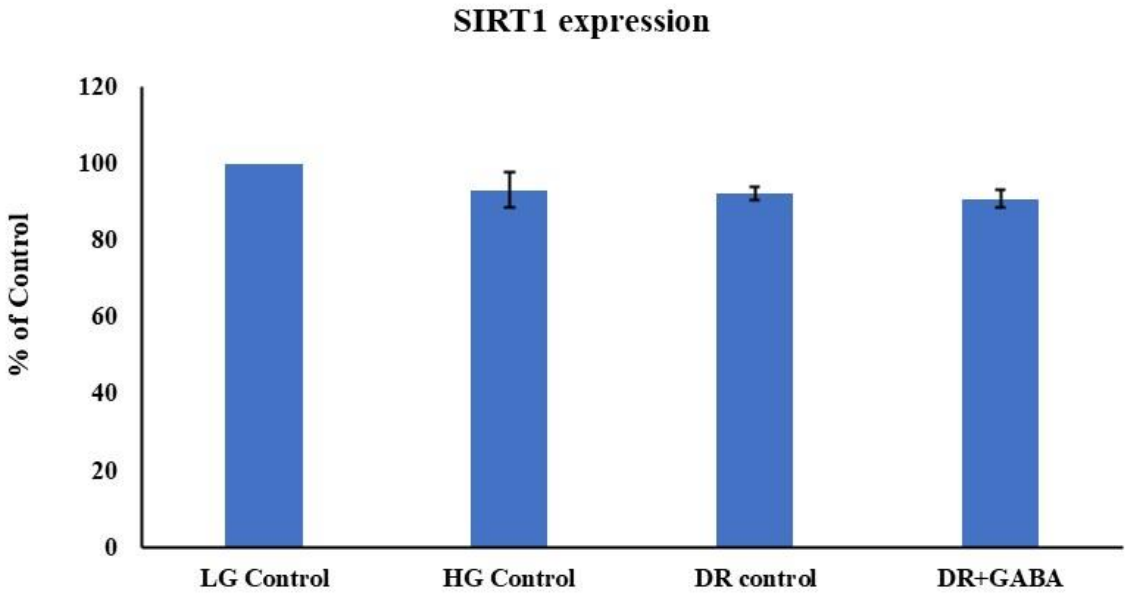


Figure 6.61: SIRT1 expression levels as determined by western blot in 3T3L-1 cell line upon treatment with UMB.

All the values are shown as mean ± S.E.M (with 3 separate blots)

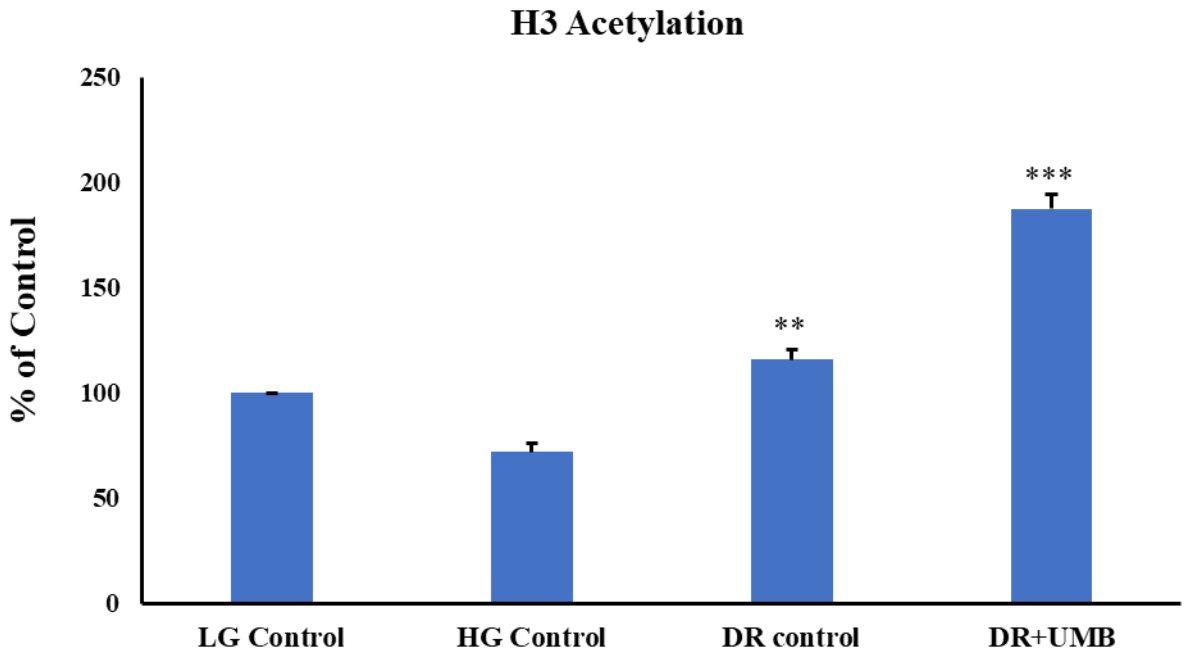


Figure 6.62: Histone 3 acetylation levels as determined by western blot in 3T3L-1 cell line upon treatment with GABA.

All the values are shown as mean ± S.E.M (with 3 separate blots), ***$p<0.001$, **$p<0.01$, and *$p<0.05$, vs control HG

Chapter 7

Discussion

Diabetes is an endocrine system disorder identified by unusually high blood glucose levels and is anticipated to afflict 693 million individuals by 2045 (Cho et al., 2018). Vascular complications including both macrovascular and microvascular (diabetic retinopathy, neuropathy, and kidney disease, or DKD) diseases cause the majority of death in people with diabetes (Morrish et al., 2001). They place a significant financial burden on society due to differences in healthcare access and costs between developed and developing nations (Fernandes et al., 2016, American Diabetes Association, 2017; Liyanage et al., 2015).

The term DN implies the presence of a characteristic pattern of glomerular disease and describes a clinical state characterized by persistent albuminuria and deterioration in renal function. According to reports, DN affects 20% to 50% of people with diabetes and causes ESRD. In the United Kingdom, DN accounts for 28% of people starting renal replacement therapy (RRT), while those numbers are 44% in the US and 38% in Australia (Byrne et al., 2018). Diabetes patients who acquire kidney disease may witness cardiovascular events, mortality, CKD progression, and ESRD (Fox et al., 2012). ACE inhibitors and ARB are being used to treat DKD, as well as blood pressure and glucose management. However, these treatments have a limited ability to stop the disease from progressing (Kanwar et al., 2011).

Epigenetic processes can change gene expression and function and facilitate interactions among genes and the environment without changing the underlying DNA sequence. Nutritional status has a significant impact on the progression of DM and DKD and these factors regulate epigenetic states, it is expected that epigenetics play an important role in the pathogenesis of DKD (Kato and Natarajan, 2014 and Keating et al., 2018).

Apart from the fact that blood glucose might be regulated with food, exercise, and drugs such as insulin, many people nevertheless suffer from a variety of life-threatening problems even after glucose levels have normalized, suggesting that target cells may retain a "memory" of previous glucose exposure that causes its harmful effects to persist long after glycaemic control has been achieved. Observations of GM have been seen in laboratory models and clinical trials (Nathan et al., 2005; de Boer et al., 2011).

Drugs like metformin have been used in treating and preventing DN but these synthetic drugs are loaded with side effects. To overcome this challenge our study focussed on choosing phytochemicals using a combined approach of in silico, in vitro, as well as in vivo studies. Scientific research suggests that two molecules with identical structures may have similar biological roles (Martin et al., 2002). Finding novel phytochemicals requires an understanding of the structures of compounds that are currently used in the treatment of GM and diabetic nephropathy. In the current work, the query structure for similarity searching was based on the natural phytochemicals selected from a thorough review of the literature.

In the present study, 6 natural epigenetic modifiers Metformin, Resveratrol, Vanillic Acid, Esculetin, Genistein, and Berberine were chosen to be used as query structures to search similar compounds. We used structurally related phytochemicals found using a Tanimoto-based 2-dimensional similarity search based on PubChem fingerprints (75%; Tanimoto 0.75 as a filter). According to Bolton et al. (2011), similar compounds can also be referred to as neighbors as 2-D neighbors and 3-D neighbors, respectively, and as "Similar Compounds" and "Similar Conformers." Two substances are deemed to be 2-D neighbors if their 2-D molecular similarity scores are 0.7 or higher. Tanimoto coefficient and binary fingerprints from the PubChem subgraph are used to determine the score.

The similar phytochemicals found were taken further for auto dock and ADME studies. Accurate beginning structures are essential for efficient molecule docking. It is crucial to receive the correct structures in the correct format from the appropriate source. Academics have thoroughly studied the SIRT1 structure. The highest-resolution structures with co-crystallized ligands and no missing or altered residues receive precedence. Each PDB ID has access to all information, including resolution and mutant residues. The Crystal structure of the SIRT1 catalytic domain bound to NAD and an EX527 analog (PDB ID-4I5I), was selected for the study and retrieved from PDB. Sirtuins are protein deacetylases that govern cellular functions such as metabolism and stress responses. They have also been linked to aging and aging-related illnesses (Michan and Sinclair, 2007; Haigis and Sinclair, 2010). Sirtuins are the only proteins that hydrolyze one NAD+ cosubstrate per deacetylation process (Sauve et al., 2006), tying their activity to cellular energy levels.

The crystal structures of the Sirtuin catalytic domains, which are 250 amino acids long and conserved, revealed that the substrates, NAD+ and acetyl-Lys of the protein substrate, enter the active site from opposite sides of a cleft. The binding of the protein substrate causes cleft closure, and NAD+ binding orders a "cofactor binding loop". Sirtuins are regarded as promising pharmacological targets for the treatment of metabolic syndrome (e.g., SIRT1 activators), cancer (e.g., SIRT1 and Sirt3 inhibitors), and neurological disorders, among others (e.g., Sirt2 inhibitors). However, scantiness in current Sirtuin inhibitors has impeded physiological research, target confirmation, and therapeutic development, as they usually have low potency and/or isoform selectivity and exploit unknown binding locations and processes (Cen, 2010 and Neugebauer et al., 2008).

Here we took protein that had EX527 analogue 35 bound to it. Upon docking 35 with the protein itself first, to validate the docking process we saw that analogue 35 binds deep within the catalytic cleft, displacing NAD (+) nicotinamide and pushing it into an extended shape. The expanded NAD (+) shape hinders substrate binding sterically. The SIRT1/NAD (+)/35 crystal structure describes a unique mode of histone deacetylase inhibition and provides a foundation for understanding and rationally optimizing drug-like molecule regulation of this therapeutically essential target. Afterward, the chosen phytochemicals were docked in the same active site with the same characteristics using the docking software Auto Dock -Vina (Trott & Olson, 2010), which is a web-based server. The results of docking were examined using the SIRT1 binding affinities (kcal/mol) for the phytochemicals (Table 6.7-6.18). According to the results, phytochemicals were chosen based on their binding

affinities (Kcal/mol), binding modes, and RMSD scores for further studies. Hence, we hypothesize that the phytochemicals with the best binding energies with the protein **SIRT1 protein (PDB ID:4I5I)** are the potent SIRT1 inhibitors as they bind at the same place where EX527 analogue binds.

Drug discovery is a resource and time-consuming process where a large number of molecules are screened on different parameters with the ultimate aim of finding those having the best chance of becoming an effective medicine. A molecule to be potent enough should show low toxicity along with high biological activity. Where its access to and concentration at the target in an organism is important as well. In reports, it has been observed that early pharmacokinetic screening of the molecule reduces the risks of pharmacokinetic-related failures in clinical phases (Hay et al., 2014). In silico models have been employed extensively where chemical structures are numerous but resources and availability of those are a problem, as an alternative for experimental procedures to predict ADME (Dahlin et al., 2015).

For a potent molecule to be effective as the drug has to reach its target site where it should remain in its bioactive form long enough for the events to occur. Development of drugs involves ADME assessment i.e. absorption, distribution, metabolism, and excretion in a stage where several compounds are numerous whereas the availability of physical samples is limited. To overcome this challenge in silico computer model act as an alternative to this. We have here, used the SwissADME web tool which is a predictive model for pharmacokinetics, drug-likeness, and physicochemical properties. In this, BOILEDegg is one important method that shows whether your compound is being GI absorbed or is BBB permeant or not. A spider web graph is a unique way of showing data across many dimensions. We did ADME studies on a few similar phytochemicals for each parent compound.

It is reported that a molecule that is soluble eases drug development processes, handling, and further formulation (Ritchie et al., 2013). For drugs targeting oral administration, solubility has always been the main concern (Ottaviani et al., 2010). Moreover, a drug that has to be given other than oral means also has to be highly soluble in water so that a precise quantity of active ingredient is delivered (Savjani et al., 2012). For skin permeation higher the negative value of log k_p lesser the molecule is permeant to the skin. The ability of the molecule to be passively absorbed in the gastrointestinal tract and BBB permeance is shown through the BOILED-Egg model (Daina and Zoete., 2016). Permeability glycoprotein (P-GP) is an important member of ATP-binding cassette transporters. It protects the CNS from xenobiotics (Szakacs et al., 2008). P-GP is also over-expressed in some cancers leading to multi-drug resistant tumors (Sharom, 2008). It is important to assess whether the molecule being substrate or non-substrate of P-GP, can actively be effluxed through biological membranes like from the GI tract to the lumen or from the brain (Montanari and Ecker., 2015).

Cytochromes P450 (CYP) are a superfamily of isoenzymes that play vital roles in the removal of drugs through metabolic biotransformation (Testa and Kraemer., 2007). Reports have highlighted that CYP and P-gp can process molecules together to provide protection to tissues (van waterschoot and Schinkel., 2011). According

to reports, nearly 50-90% of molecules serve as a substrate for 5 major CYP isoforms (CYP2C19, CYP1A2, CYP2D6, CYP3A4, CYP2C9) (Wolf and Smith., 2000 and Di et al., 2014). So inhibiting these enzymes is an important parameter of drug-drug interactions (Hollenberg., 2002 Huang et al., 2008) and leads to toxic and unwanted effects because of the lower clearance and accumulation of the drug metabolites (Kirchmair et al., 2015). Since many inhibitors of CYP isoforms have been identified, many affect CYP while others show selectivity for specific isoenzymes, it is hence necessary to predict the probability by which molecules will cause drug interactions by inhibiting CYP. So SwissADME helps in predicting whether the molecule will be a substrate of P-gp or inhibit the CYP. Based on all these parameters we chose two compounds **UMB** and **GABA** to study in vitro and in vivo.

We saw UMB's ability to scavenge DPPH radicals. The stable DPPH radical has been extensively employed to assess substances' potential to behave as scavengers of free radicals or hydrogen donors, and hence to assess antioxidant activity. (Nenadis and Tsimidou, 2002). At 107µg/mL, UMB was found to have potent DPPH radical-scavenging properties while GABA at 141.09 µg/mL had potent DPPH radical-scavenging properties. UMB has the potential to operate as an electron donor, interacting with free radicals to transform them into more stable byproducts and stop the chain reaction of radicals. Among natural antioxidants, UMB is slightly lipophilic, but it is more hydrophilic than α-tocopherol (Kaneko et al., 2003). Since UMB is thought to localize near the membrane surface as flavonoids do, it has the ability to scavenge free radicals (Torres et al., 2006). The discovery of UMB's free radical-scavenging action motivated us to investigate its nephroprotective potential in experimental model systems. In this study, we show that UMB efficiently shields 3T3L-1 cells from potentially fatal ROS and extends the lives of lethally exposed cells. UMB did not cause any cytotoxicity in 3T3L-1 cells up to 199µM concentration analyzed by cell proliferation assay.

GABA released from β-cells can act on GABA receptors hence suppressing glucagon secretion (Rorsman et al.,1989; Xu et al., 2006). GABA was first identified as a constituent of tuber tissue in potatoes (Steward., 1949). It was discovered in the mammalian brain in 1950 by Roberts and Franklin (Roberts and Frankel., 1950). In 1960, Roberts and Heidelbergin revealed its role as a neurotransmitter (Roberts and Eidelberg., 1960). GABA is also used in food products like gammalone, gabaron tea, shochu, and cheese (Sawai et al.,2001, Yokoyama et al., 2002). It improves blood plasma concentration, protein synthesis, and growth hormones in the brain (Cho et al., 2007). It is known to have diuretic, anti-depressive, hypotensive, tranquilizing, antioxidant, and antidiabetic effects and regulates growth hormone secretion (Ueda et al., 2007). GABA was also effective in treating neurological disorders like Parkinson's and Huntington's (Okada et al., 2000). Hagiwara et al., 2004 demonstrated that GABA acts as a strong secretor of insulin from the pancreas, therefore preventing diabetes. It is also reported that foods enriched in GABA foods can inhibit cancer proliferation (Park and Oh., 2007). As a result, it is classed as a bioactive constituent in food and medicinal products (Kim et al., 2009). In our study, on the 3T3L-1 cell line, EC_{50} of GABA was found at 145µM.

We proceeded further to check the oxidative biomarkers and the effect of UMB and GABA on the diabetic model and GM model. 3T3L-1 cell lines were employed in the current work to simulate hyperglycemic and GM conditions. According to Halliwell & Gutteridge (2015), hyperglycemia results in increased oxidative stress, which damages cells. According to Ceriello et al. (2003), there is a decline in oxidative defense in pre-diabetic conditions without complications. Increased lipid peroxidation, an unbalanced glutathione redox state, and a decline in the activity of antioxidant enzymes are only a few of the symptoms. These modifications imply that hyperglycemia drives oxidative stress that eventually results in diabetes and related disorders. According to Pandey et al. (2010), excessive glucose levels promote OFRs production, which weakens the body's defenses resulting in oxidative stress. According to Negre-Salvayre et al. 2009, insulin resistance is assumed to be governed by oxidative stress, which leads to glucose intolerance, which in turn causes an increase in microvascular and macrovascular problems.

Lipids are the main target of ROS, leading to their ability to peroxidation (Hematyar et al., 2019). According to Guo et al, 2012, ROS-induced peroxidation of lipids results in the formation of MDA as well as other highly reactive aldehyde molecules. According to Lobo et al. (2010), LPO produces hydrogen peroxides by extracting the hydrogen atom from the lipids with the aid of peroxyl radicals, which spread the free radical pathway even further. The consequences of diabetes are to blame for the rise in LPO levels. Scientific studies demonstrate that rat models of insulin resistance or T2DM have indicators of increased LPO. In the current study, we tested the GABA and UMB in 3T3L-1 cell lines for their ability to ameliorate GM and anti-diabetic effects. The results demonstrated that increased oxidative stress makes the lipids of the hyperglycemic control more vulnerable to peroxidation. A biomarker called TBARS is used to gauge fat degradation brought on by LPO (Ghani et al., 2017). When compared to the positive control, the negative control and the DR control had higher amounts of TBARS. However upon treatment with GABA and UMB, the increased TBARS level decreased but when GABA and UMB were supplemented along with DR it further decreased the TBARS levels.

Through both nonenzymatic protein glycation and auto-oxidation of glucose, prolonged hyperglycemia in diabetes increases their synthesis of oxygen free radicals (OFRs). MDA is created when OFRs have a cytotoxic effect on membrane phospholipids. Membrane peroxidation causes a loss of membrane integrity while increasing fluidity and permeability (Feng et al., 2022). Cells are shielded from oxidative damage by antioxidant enzymes like GST, peroxidase, catalase and SOD (Iova et al., 2021). Enzymatic and nonenzymatic defense mechanisms exist to safeguard the biological system from oxidative damage. The most researched antioxidant enzymes are glutathione peroxidase (GPx), SOD, and catalase (CAT), whereas the most researched nonenzymatic antioxidant substances are ascorbic acid and glutathione (GSH) (Giacco et al., 2010). SOD protects the body by catalyzing the dismutation of the extremely reactive superoxide ions into mol O2 and less harmful hydrogen peroxide, the reaction serves as a vital defense mechanism against oxidative damage (Yang et al., 2022). It is well known that diabetic hyperglycemia generates a significant amount of superoxide, which harms the kidneys. The overall accumulation of superoxide anion is controlled by a balance between superoxide

anion formation and antioxidant capacity. An increase in the glucose flow promotes superoxide anion production by the mitochondrial ETC. SOD has a function in the pathogenesis of diabetic nephropathy. Several investigations found a decrease in SOD in diabetic tissue and blood (Shukla et al., 2012).
Glutathione-S-transferases (GSTs) are crucial for the metabolism of xenobiotics, detoxification, and the reduction of hydrogen peroxide. Additionally, glutathione thiol group conjugation to electrophilic xenobiotics protects against cellular damage brought on by toxicants by shielding cells from the impacts of the substances' toxic effects. According to Zhang et al. (2021), GST activity was present in the majority of mammalian organs, typically the liver, which is crucial for detoxification. According to Sailaja et al. (2003), patients with T2DM had lower levels of GST activity in their reticulocytes and erythrocytes compared to controls.

High levels of H2O2 have been connected to catalase deficiency. These increased amounts of H_2O_2 cause pancreatic cell injury, which disrupts insulin secretion and signaling and changes glucose metabolism, which causes diabetes. Additionally, diabetic patients have been found to have reduced catalase activity. According to Patel et al. (2013), gene expression fingerprint investigations conducted during the research of functional alterations brought on by hyperglycemia showed increased hydrogen peroxide production while also downregulating CAT gene expression. These reports make it clear that high glucose causes low catalase levels because it produces too many hydrogen peroxide species, which causes oxidative stress and advances T2DM. In this study, we also checked GABA and UMB in antioxidant defense mechanisms. There was a decrease in catalase, GST, and SOD levels in hyperglycemic control and DR control. But upon treatment with GABA and UMB along with DR, we saw an increase in these levels when compared to positive control.

In this study, UMB and GABA were taken further for in vivo studies, as they proved to be a good antioxidant, anti-diabetic, and ameliorated GM. Current treatment methods for diseases like DN are centered on synthetic drugs that are rather expensive. These synthetic drugs have adverse effects on different parts of the body. They can also alter metabolic pathways in the human body. Hence there is a need for a cost-effective and non-toxic method for the treatment of diseases and their progression. Our previous in silico analysis highlighted that bioactive phytochemicals like UMB and GABA could possess the potential to cure such diseases. UMB is known to possess anti-bacterial, anti-fungal, and antioxidant activity but its ability to ameliorate GM is still unknown. Furthermore, animal models hold an essential position in research to study human diseases.

We developed an animal model that mimicked GM and DN conditions by feeding rats on HFD for a duration of 16 weeks and then reversing their diet for the consecutive 8 weeks, resulting in increased blood glucose, body weight, cholesterol, kidney, and liver function tests in HFD groups. In line with prior research (Tallapragada et al., 2014 and Karpe et al., 2014;) During 8, 16, and 24 weeks, plasma glucose rose evidently, as body weight, triglycerides, and cholesterol in the animals feeding on HFD. The increased rate of body weight growth was exacerbated in HFD-fed rats after 6 weeks. This suggests that insulin resistance gradually developed under hyperlipidemic settings following a HFD. Even after the duration of 8 weeks of diet reversal, the reversal group gained the same amount of weight as the HFD group, showing that the previous phase of HFD feeding has left

its imprint. GABA and UMB were proven to be helpful in lowering increased body weight starting from the very first week itself. The prevalence of GM was demonstrated by the fact that diet reversal had no impact on the hyperglycemia generated by HFD. GABA and UMB, on the other hand, reduced the increased glucose, lipids, and cholesterol.

Diabetes-induced renal impairment is exacerbated by urine microalbumin excretion, and increased BUN and creatinine levels (Saravanan and Pari, 2016, and Viswanathan et al., 2004;). BUN, creatinine, and uric acid levels were observed to be higher in the HFD-fed group, however, treatment with GABA and UMB reversed this. Plasma BUN and creatinine levels increased at the beginning of the 16th week of HFD feeding, indicating renal anomalies. Dietary changes had no effect on the renal function markers. GABA and UMB, on the other hand, improved renal impairment by lowering plasma urea and creatinine levels. Diet reversal was ineffective in combating the aberrations caused by HFD eating, but it could decrease them when combined with UMB and GABA. H&E examination of HFD-fed kidney cross-sections indicated increased glomerular size and glomerular space (glomerular tuft area). Diet reversal alone did not ameliorate these HFD-induced abnormalities, but it did when combined with GABA and UMB.

For the first time, we demonstrate that GABA and UMB block the GM which leads to the advancement of HFD-induced kidney damage. It was previously reported, that GM is found to be responsible for HFD-induced renal and vascular endothelial impairment (Kumar et al., 2016, Tallapragada et al., 2015). In line with earlier findings, feeding rats on a HFD for 16 weeks developed insulin resistance, which is evidenced by high blood sugar, cholesterol, and triglyceride. (Bendale et al., 2013 and Karpe et al., 2012).High-fat diet-fed Sprague Dawley rats are frequently employed as a model for human type II diabetes (Hsu et al., 2016 and Soler et al., 2012). Increased creatinine and BUN levels after 16 weeks of HFD eating suggested the development of renal impairment. Eight weeks of constant DR decreased cholesterol levels but did nothing to address hyperglycemia or renal impairment. However, a combination of diet reversal and phytochemical treatment successfully recovered kidney function along with insulin sensitivity. Our in vivo model closely resembles the clinical scenario where insulin resistance-related high-calorie, HFD results in kidney problems. Due to the fact that there was no change in the albumin levels in the plasma, it is early-stage nephropathy (data not shown). At the molecular, microscopic, biochemical, and macroscopic levels, the HFD animals were unable to respond to DR after the initial exposure to hyperglycemia and hypertriglyceridemia, demonstrating the emergence of GM. We report that, after the duration of 16 weeks of HFD feeding, 8 weeks of DR did not reverse the insulin resistance, but that, when combined with phytochemical treatment for 8 weeks, the abnormalities of insulin resistance are noticeably improved. Treatment with GABA (100 and 200 mg/kg/day) and UMB (20 and 40 mg/kg/day) prevents GM brought on by HFD and restores renal and liver problems.

As previously described, the histology of the kidney showed structural changes due to the HFD diet, like enlarged glomeruli and Bowman's capsular gap (Deij et al., 2009). Even after changing our diets, we still saw identical tiny maladaptations in the kidneys. There was no apparent advantage of diet reversal because of the degree of kidney impairment following initial HFD feeding, both groups had comparable results.

ROS has an important role in DN (Gujjala et al., 2016). Management of antioxidants presents a novel opportunity. Antioxidant management can be a good option for managing diabetes. In this study, UMB and GABA considerably reduced the ROS production in HFD-fed rat's kidneys. In line with ROS creation, GABA and UMB along with DR dramatically decreased the generation of MDA in the kidneys of HFD-fed rats. ROS are generated because of the electron leakage into oxygen from numerous systems in our bodies, and endogenous antioxidant enzymatic defense is crucial for neutralizing ROS. A decrease in the efficacy of antioxidant defense mechanisms might worsen the adversity of OFRs-induced oxidative damage (Salvemini and Cuzzoreca, 2003; Singh et al., 2000). Several studies demonstrate that antioxidants can protect against free radical generation.

In this work, we found that HFD-induced diabetic rats had lower SOD, GST, and catalase activity. The initial line of cellular defense against oxidative damage is SOD and catalase, which break down superoxide radicals and H2O2 before combining to form the more harmful hydroxyl radical. SOD eliminates superoxide radicals (O2) in the cytoplasm and mitochondria, and the resulting H2O2 is removed by catalase. The superoxide radical produced at the site of nephropathy affects SOD and catalase, resulting in the inactivation of these enzymes and the accumulation of superoxide anion, which destroys the kidney. In our study, upon DR and treatment with the phytochemicals UMB and GABA, the decreased levels of GST, SOD, and catalase returned to near normal as compared to the vehicle control group.

Reduced glutathione (GSH) is a crucial antioxidant that protects the body from free radical damage. GSH deficiency reduces the efficacy of glutathione-dependent enzymes such as GPx and GST in HFD-induced diabetic rats (Zhou et al.., 2018). The activities of GST were investigated in this study and found that GST level was diminished in the kidney of diabetic rats and DR alone could not ameliorate the decreased GST levels but upon DR and treatment with UMB and GABA GST levels were restored to near normal.

Several clinical care techniques are indicated for ameliorating DN (Brownlee, 2005). Even with good multifactorial therapy, ESRD develops fast in people having DN and GM. As a result, innovative therapeutic drugs are required to manage DN. A study found that activated sirtuin 1 (SIRT1) protects against kidney impairment in high blood glucose situations where resveratrol, reduced the TGF-1-induced elevation of SMA, and fibronectin in mice with unilateral ureteral obstruction. (Li et al., 2010). SIRT1 inhibitors, on the other hand, also protect against diabetes-induced kidney fibrosis. EX-527, a novel SIRT1 inhibitor, limits interstitial fibroblast activation in renal interstitial fibroblast cell line and reduced the expression of fibroblast activation markers (Ponnusamy et al., 2014, 2015). SIRT1 inhibitors have antifibrotic properties that are linked to numerous receptors like EGFR and PDGFR, and various intracellular signaling pathways. Renal fibrosis advances as the phosphorylation levels of EGFR and PDGFR increase, and in animal models, inhibiting these receptors has been shown to lower kidney fibrosis activation. (Liu et al., 2011 and 2012). This study illustrated that both phytochemicals are SIRT1 inhibitors and protect against DN and GM in the SD rat model induced by HFD feeding.

This is the first study to show that UMB and GABA prevent the progression of HFD-induced renal impairment and reverse the GM condition caused by hyperglycemia. SIRT1 expression is known to alter under many physiological and pathological circumstances. SIRT1 is generally reduced in oxidative stress, which causes ESRD, micro and macrovascular complications. Some studies have shown that resveratrol and SRT1720 have a variety of off-target effects and do not directly target SIRT1 (Pacholec et al., 2010). It was discovered that activating SIRT1 with SRT1720 caused fibrosis in the kidney. These findings revealed that SIRT1 has importance in inhibiting renal fibrogenesis (Ponnusamy et al, 2015).

In this study, we also attempted to explain the role of post-translational histone modification under in vitro hyperglycemic settings, as well as the effect of UMB and GABA on post-translational modification under such conditions. In 2010, Bonasio and colleagues showed that post-translational changes, such as histone alterations, have a significant role in maintaining the normal pattern of cellular transcription. According to Gupta et al. (2010), T2DM is caused by hyperglycemia/hyperinsulinemia, which affects the pattern of post-translational histone modifications and, as a result, causes dysregulation of gene expression, which eventually contributes to the progression of diabetes.

HAc regulates eukaryotic gene transcription. HAT and HDAC regulate HAc in vivo. According to Shvedunova and Akhtar, 2022, there is a dynamic balance between HAT and HDAT activities that regulate gene activation and repression. The mechanisms underlying H3 acetylation and transcription are unknown. We used the 3T3L-1 cell line after UMB and GABA treatment to examine changes in histone H3 acetylation (H3ac) and SIRT1 levels under hyperglycemic and diet reversal conditions. The current study found that under high glucose/hyperglycemic conditions, H3Ac at lysine9/14 residue (K9/14) was lower than under normal glucose conditions. According to Kabra et al., 2009, increased acetylation of H3 is induced by insulin in a time-dependent manner under normal glucose conditions, whereas hypoacetylation is seen under high glucose conditions, implying that changes in glucose level can disrupt the homeostasis of acetylation, affecting the PTMs of H3. According to Gaikwad et al. (2010), hyperinsulinemia or insulin resistance causes a decrease in the acetylation of H3 in the kidney and an increase in acetylation in the heart. Yet, in hyperglycemic or high glucose situations, there is a decrease in the acetylation of H3 in both the heart and the kidneys.

Oxidative stress is caused by high glucose levels in cells. Because oxidative stress affects HAc, our findings in this study imply that under hyperglycemic settings, there is a decrease in the acetylation of H3at k9/14. Furthermore, when compared to the untreated hyperglycemic group, treatment with GABA and UMB reversed hypoacetylation and elevated H3ac. But when the SIRT1 expression was studied we found no change in the expression. This may be because the treatment given overnight was not sufficient to modify the expression of SIRT1. A similar result was also seen in the SIRT1 assay as it also did not show any change in different groups.

In our study, we observed decreased SIRT1 expression in HFD-fed rats when compared to the control rats as observed by the SIRT1 assay. Diet reversal as well as phytochemical treatment did not change the expression

of SIRT1 in the kidneys of these rats. This may be because the phytochemicals GABA and UMB have no effect on the protein expression of SIRT1 but can inhibit its activity. As a result, determining the functional role of SIRT1 in tissue fibrosis is critical in order to use more selective inhibitors. It could be related to their vague features. However, the role of SIRTs in tissue-specific fibrosis modulation is still debated. We cannot rule out the likelihood of UMB and GABA being SIRT1 inhibitors as they played a role in the prevention of renal fibrogenesis in our investigation. Our data allow us to draw the conclusion that short-term diet reversal cannot restore the difficulties brought on by insulin resistance due to GM. To determine the involvement of GABA and UMB in the epigenetic changes causing GM prevention, more research is needed. We hypothesized that GABA and UMB may give protection against HFD-induced renal fibrosis in SD rats by modifying oxidative stress, blood glucose, lipid, renal, and liver profiles. **After observing the findings, we reject the null hypothesis.**

Our findings are beneficial to patients with DN as understanding the experiences, challenges, and needs of individuals living with diabetic nephropathy is crucial. Endocrinologists and Nephrologists are the specialists who are present at the forefront of managing diabetic nephropathy. They can benefit from this research by gaining insights into the long-term effects of glycemic control on kidney function. This information may inform clinical decision-making and treatment approaches. Researchers and scientists are experts in the field of diabetes, nephrology, and related disciplines and our research may advance the scientific understanding of the relationship between glycemic memory and diabetic nephropathy. Since our research identifies novel mechanisms or targets related to glycemic memory and diabetic nephropathy, it may have implications for drug development. Pharmaceutical companies could use this information to create more effective treatments. This could lead to further research, potentially uncovering new therapeutic targets or interventions. Pharmaceutical companies could use this information to create more effective treatments.

The future scope of the study includes the toxicity profiles of the phytochemicals and dosage study. The future scope of the study is to further evaluate tissue fibrosis and the role of inflammation in the pathogenesis of GM and DN. Exploration of selective SIRT1 inhibitor to develop more targeted therapeutic interventions for diabetes-related insults. In-depth investigation of the GABA and UMB underlying mechanisms through which they exert their activity in SIRT1 activity, oxidative stress, blood glucose, lipid profiles and other parameters. Delving into the epigenetic modifications induced by GABA and UMB and how these changes relate to the prevention of metabolic disorders like insulin resistance and associated complications. Transitioning the findings from preclinical models (rats) to clinical studies to assess the potential therapeutic applications of UMB and GABA in humans. Disseminating research findings through peer-reviewed journals, conferences, and other platforms to contribute to the broader scientific community's understanding of these topics.

Chapter 8

Conclusion

DN is the most common and severe consequence of DM, with increased mortality in T2DM individuals. Nearly 80% of people worldwide currently utilize herbal plants to treat a variety of illnesses, according to the WHO. Only a small number of plants, whose mechanisms of action are unknown, have been discovered to cure diabetes-related issues. The most often given treatments for T2DM today include metformin, however, these medications have a variety of side effects. As a result, researchers are focusing on identifying novel natural phytochemicals in order to reduce toxicity while improving efficacy.

We chose GABA and UMB to study after extensive data mining and similarity searching. *In silico* molecular docking studies revealed that GABA and UMB had a strong binding affinity with SIRT1 on the same scale as standard drug metformin and also showed binding in the same active site as metformin determined by its amino acid residue interaction profile, indicating that GABA and UMB is a potential SIRT1 modulator. GABA and UMB's in-silico ADME qualities revealed its solubility in water, high GI absorption, penetration to the BBB, and good bioavailability, indicating optimum bioavailability.

GABA and UMB demonstrated strong scavenging action in a dose-dependent manner in the DPPH free radical analysis experiment, indicating that it is a good antioxidant molecule. The cell viability assay (MTT assay) was performed to screen GABA and UMB for its toxicity on cell proliferation of normal cells (3T3L-1cell line) and found that GABA and UMB is non-toxic in vivo. Under hyperglycemic conditions, GABA and UMB reduced oxidative stress by elevating the SOD, catalase, and GST, as well as lowering LPO in a dose-dependent manner, indicating their potential in reducing oxidative stress by reducing free radical formation. Free radicals produced by oxidative stress are known to cause pancreatic-cell destruction, leading to an imbalance in insulin secretion and signaling, as well as altering the glucose metabolic process, resulting in the onset of diabetes. Our findings suggested that GABA and UMB are good antioxidants capable of repairing injured pancreatic cells and insulin secretion. In vitro results implied that GABA and UMB were able to ameliorate the GM condition and were good anti-diabetic compounds.

We successfully created a Sprague-Dawley rat model of HFD-induced metabolic dysfunction of glucose and kidney damage in-house. This is the first study to show that UMB and GABA protect against HFD-induced renal fibrosis via the oxidative stress route, which plays an important role in renal fibrosis. Similarly to other recent discoveries of SIRT1 inhibitors, in a well-defined animal model, blocking SIRT1 with EX527 analogues and structurally similar compounds lowers the incidence of kidney fibrosis. Furthermore, the constructive effect of GABA and UMB may be connected with the inhibition of LPO and increasing other oxidative biomarkers. The in vivo study done on SD rats also concluded that GABA and UMB were successfully able to ameliorate the GM and DN condition caused due to HFD. GABA and UMB not only ameliorated DN and GM but also decreased the increased body weight and plasma glucose level. GABA and UMB like synthetic drugs didn't

have any side effects proving them a good alternative to synthetic drugs. Although its ability to modulate SIRT1 on the epigenetic axis is yet to be fully elucidated. Further in-depth testing of these compounds can be helpful for pharmaceutical companies to develop a drug with no or least side effects and can increase the life of diabetic patients.

Essentially, our findings are the first to provide direct evidence that SIRT1 inhibitors may play a significant role in ameliorating glycemic memory. Our findings provide a comprehensive understanding of SIRT1's (patho-)physiological involvement in nephropathy and GM. These data can be used to create a new DN treatment plan. As a result, our data suggest the potential clinical applicability of GABA and UMB as renoprotective medications in HFD-induced diabetes patients. Further research is needed to establish the mechanism underlying SIRT1 inhibition associated with renal fibroblast activation and proliferation.

Bibliography:

Adler, A. I., Stevens, R. J., Manley, S. E., Bilous, R. W., Cull, C. A., Holman, R. R., & UKPDS Group. (2003). Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney international*, *63*(1), 225-232.

Aebi, C., Lafontaine, E. R., Cope, L. D., Latimer, J. L., LUMBley, S. L., McCracken Jr, G. H., & Hansen, E. J. (1998). Phenotypic effect of isogenic uspA1 and uspA2 mutations on Moraxella catarrhalis 035E. *Infection and immunity*, *66*(7), 3113-3119.

Aittoniemi, J., Fotinou, C., Craig, T. J., de Wet, H., Proks, P., & Ashcroft, F. M. (2009). SUR1: a unique ATP-binding cassette protein that functions as an ion channel regulator. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1514), 257-267.

Al-Amin, Z. M., Thomson, M., Al-Qattan, K. K., Peltonen-Shalaby, R., & Ali, M. (2006). Anti-diabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin-induced diabetic rats. *British journal of nutrition*, *96*(4), 660-666.

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*, *17*(8), 487-500.

ALTamimi, J. Z., AlFaris, N. A., Al-Farga, A. M., Alshammari, G. M., BinMowyna, M. N., & Yahya, M. A. (2021). Curcumin reverses DNin streptozotocin-induced diabetes in rats by inhibition of PKCβ/p66Shc axis and activation of FOXO-3a. *The Journal of nutritional biochemistry*, *87*, 108515.

American Diabetes Association. (2018). Economic costs of diabetes in the US in 2017. *Diabetes care*, *41*(5), 917-928.

Assmann, T. S., Recamonde-Mendoza, M., de Souza, B. M., Bauer, A. C., & Crispim, D. (2018). MicroRNAs and diabetic kidney disease: Systematic review and bioinformatic analysis. *Molecular and cellular endocrinology*, *477*, 90-102.

Atlas, D. (2015). International diabetes federation. *IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation*, *33*, 2.

Augustin, A. J., Breipohl, W., Böker, T., Lutz, J., & Spitznas, M. (1993). Increased lipid peroxide levels and myeloperoxidase activity in the vitreous of patients suffering from proliferative diabetic retinopathy. *Graefe's archive for clinical and experimental ophthalmology*, *231*(11), 647-650.

Babaei-Jadidi, R., Karachalias, N., Ahmed, N., Battah, S., & Thornalley, P. J. (2003). Prevention of incipient DNby high-dose thiamine and benfotiamine. *Diabetes*, *52*(8), 2110-2120.

Bakris, G. L., Fonseca, V. A., Sharma, K., & Wright, E. M. (2009). Renal sodium–glucose transport: role in DM and potential clinical implications. *Kidney international*, *75*(12), 1272-1277.

Bakris, G. L., Toto, R. D., McCullough, P. A., Rocha, R., Purkayastha, D., Davis, P., & GUARD (Gauging Albuminuria Reduction With Lotrel in Diabetic Patients With Hypertension) Study Investigators. (2008). Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. *Kidney international*, *73*(11), 1303-1309.

Bakris, G., Viberti, G., Weston, W. M., Heise, M., Porter, L. E., & Freed, M. I. (2003). Rosiglitazone reduces urinary albumin excretion in type II diabetes. *Journal of human hypertension*, *17*(1), 7-12.

Ban, C. R., & Twigg, S. M. (2008). Fibrosis in diabetes complications: pathogenic mechanisms and circulating and urinary markers. *Vascular health and risk management*, *4*(3), 575-596.

Barlow, S. E., & Expert Committee. (2007). Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics*, *120*(Supplement_4), S164-S192.

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *cell*, *116*(2), 281-297.

Bartel, D. P. (2009). MicroRNAs: target recognition and regulatory functions. *cell*, *136*(2), 215-233.

Baylin, S. B., & Jones, P. A. (2011). A decade of exploring the cancer epigenome—biological and translational implications. *Nature Reviews Cancer*, *11*(10), 726-734.

Bechtel, W., McGoohan, S., Zeisberg, E. M., Müller, G. A., Kalbacher, H., Salant, D. J., ... & Zeisberg, M. (2010). Methylation determines fibroblast activation and fibrogenesis in the kidney. *Nature medicine*, *16*(5), 544.

Bell, C. G., Teschendorff, A. E., Rakyan, V. K., Maxwell, A. P., Beck, S., & Savage, D. A. (2010). Genome-wide DNAMeanalysis for DNin type 1 DM. *BMC medical genomics*, *3*(1), 33.

Bendale, D. S., Karpe, P. A., Chhabra, R., Shete, S. P., Shah, H., and Tikoo, K. (2013) 17-_ Oestradiol prevents cardiovascular dysfunction in postmenopausal metabolic syndrome by affecting SIRT1/AMPK/H3 acetylation. *Br. J. Pharmacol*. 170, 779–795

Bera, A., Das, F., Ghosh-Choudhury, N., Mariappan, M. M., Kasinath, B. S., & Ghosh Choudhury, G. (2017). Reciprocal regulation of miR-214 and PTEN by high glucose regulates renal glomerular mesangial and proximal tubular epithelial cell hypertrophy and matrix expansion. *American Journal of Physiology-Cell Physiology*, *313*(4), C430-C447.

Bhagat, N., & Zarbin, M. A. (2019). Epidemiology, risk factors, and pathophysiology of diabetic retinopathy. In *Clinical Strategies in the Management of Diabetic Retinopathy* (pp. 1-19). Springer, Cham.

Bhatt, K., Lanting, L. L., Jia, Y., Yadav, S., Reddy, M. A., Magilnick, N., ... & Natarajan, R. (2016). Anti-inflammatory role of microRNA-146a in the pathogenesis of diabetic nephropathy. *Journal of the American Society of Nephrology*, *27*(8), 2277-2288.

Bianchi, C., Miccoli, R., & Del Prato, S. (2013). Hyperglycemia and vascular GM: truth or fiction?. *Current diabetes reports*, *13*(3), 403-410.

Bolton, W. K., Cattran, D. C., Williams, M. E., Adler, S. G., Appel, G. B., Cartwright, K., ... & Spinowitz, B. S. (2004). Randomized trial of an inhibitor of formation of AGEs in diabetic nephropathy. *American journal of nephrology*, *24*(1), 32-40.

Bosman, D. R., Winkler, A. S., Marsden, J. T., Macdougall, I. C., & Watkins, P. J. (2001). Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. *Diabetes care*, *24*(3), 495-499.

Brasacchio, D., Okabe, J., Tikellis, C., Balcerczyk, A., George, P., Baker, E. K., ... & El-Osta, A. (2009). Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. *Diabetes*, *58*(5), 1229-1236.

Brennan, E. P., Ehrich, M., O'Donovan, H., Brazil, D. P., Crean, J. K., Murphy, M., ... & Maxwell, A. P. (2010). DNAMeprofiling in cell models of diabetic nephropathy. *Epigenetics*, *5*(5), 396-401.

Brenner, B. M., Cooper, M. E., De Zeeuw, D., Keane, W. F., Mitch, W. E., Parving, H. H., ... & Shahinfar, S. (2001). Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *New England journal of medicine*, *345*(12), 861-869.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, *414*(6865), 813-820.

Brownlee, M., 2005. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54, 1615–1625.

Byrne, C., Caskey, F., Castledine, C., Davenport, A., Dawnay, A., Fraser, S., & Williams, A. (2018). 20th annual report of the renal association. *Bristol, UK: UK Renal Registry*, *10*, 000490957.

Campos, E. I., & Reinberg, D. (2009). Histones: annotating chromatin. *Annual review of genetics*, *43*.

Cao, R., Fang, D., Wang, J., Yu, Y., Ye, H., Kang, P., ... & Gao, Q. (2019). ALDH2 overexpression alleviates high glucose-induced cardiotoxicity by inhibiting NLRP3 inflammasome activation. *Journal of Diabetes Research*, *2019*.

Cen Y (2010) Sirtuins inhibitors: The approach to affinity and selectivity. Biochim Biophys Acta 1804(8):1635–1644.

Ceol, M., Gambaro, G., Sauer, U., Baggio, B., Anglani, F., Forino, M., ... & Schleicher, E. D. (2000). Pathophysiology of Renal Disease-Glycosaminoglycan Therapy Prevents TGF-β1 Overexpression and Pathologic Changes in Renal Tissue of Long-Term Diabetic Rats. *Journal of the American Society of Nephrology*, *11*(12), 2324-2336.

Ceriello, A. (2003). New insights on oxidative stress and diabetic complications may lead to a ‘‘causal’’ antioxidant therapy. Diabetes Care, 26, 1589–1596.

Ceriello, A., Ihnat, M. A., & Thorpe, J. E. (2009). The “GM”: is more than just tight glucose control necessary to prevent diabetic complications?. *The Journal of Clinical Endocrinology & Metabolism*, *94*(2), 410-415.

Chalmers, J., & Cooper, M. E. (2008). UKPDS and the legacy effect. *New England Journal of Medicine*, *359*(15), 1618-1620.

Chen, H. L., Wan, Y. G., Zhao, Q., Huang, Y. R., Shi, X. M., Meng, X. J., & Yao, J. (2013). Regulative mechanism of renal inflammatory-related p38MAPK signaling pathway in DNand interventional effects of Chinese herbal medicine. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica*, *38*(14), 2268-2272.

Chen, H., Li, J., Jiao, L., Petersen, R. B., Li, J., Peng, A., ... & Huang, K. (2014). Apelin inhibits the development of DNby regulating HAc in Akita mouse. *The Journal of physiology*, *592*(3), 505-521.

Chen, H.W., Yang, M.Y., Hung, T.W., Chang, Y.C., Wang, C.J., 2019. NelUMBo nucifera leaves extract attenuate the pathological progression of DNin highfat diet-fed and streptozotocin-induced diabetic rats. J. Food Drug Anal. 27, 736–748

Chen, L., Cui, Y., Li, B., Weng, J., Wang, W., Zhang, S., ... & Huang, Q. (2020). AGEs induce immature angiogenesis in in vivo and ex vivo mouse models. *American Journal of Physiology-Heart and Circulatory Physiology*, *318*(3), H519-H533.

Chen, L., Magliano, D. J., & Zimmet, P. Z. (2012). The worldwide epidemiology of type 2 DM—present and future perspectives. *Nature reviews endocrinology*, *8*(4), 228-236.

Chen, P. J., Furuse, J., Han, K. H., Hsu, C., Lim, H. Y., Moon, H., ... & Yeo, W. (2010). Issues and controversies of hepatocellular carcinoma-targeted therapy clinical trials in Asia: experts' opinion. *Liver International*, *30*(10), 1427-1438.

Chen, S., Jiang, H., Wu, X., & Fang, J. (2016). Therapeutic effects of quercetin on inflammation, obesity, and type 2 diabetes. *Mediators of inflammation*, *2016*.

Chen, Z. X., & Riggs, A. D. (2011). DNAMe and demethylation in mammals. *Journal of Biological Chemistry*, *286*(21), 18347-18353.

Chen, Z., Li, S., Subramaniam, S., Shyy, J. Y. J., & Chien, S. (2017). Epigenetic regulation: a new frontier for biomedical engineers. *Annual review of biomedical engineering*, *19*, 195-219.

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. I. D. F. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes research and clinical practice*, *138*, 271-281.

Cho, Y. R., Chang, J. Y., & Chang, H. C. (2007). Production of gamma-aminobutyric acid (GABA) by Lactobacillus buchneri isolated from kimchi and its neuroprotective effect on neuronal cells. *Journal of Microbiology and Biotechnology*, *17*(1), 104-109.

Comper, W. D., Osicka, T. M., Clark, M., MacIsaac, R. J., & Jerums, G. (2004). Earlier detection of microalbuminuria in diabetic patients using a new urinary albumin assay. *Kidney international*, *65*(5), 1850-1855.

Cooper, A., Mikhail, A., Lethbridge, M. W., Kemeny, D. M., & Macdougall, I. C. (2004). Pentoxifylline improves hemoglobin levels in patients with erythropoietin-resistant anemia in renal failure. *Journal of the American Society of Nephrology*, *15*(7), 1877-1882.

Cooper, M. E., & El-Osta, A. (2010). Epigenetics: mechanisms and implications for diabetic complications. *Circulation research*, *107*(12), 1403-1413.

Cordero-Herrera, I., Chen, X., Ramos, S., & Devaraj, S. (2017). (−)-Epicatechin attenuates high-glucose-induced inflammation by epigenetic modulation in human monocytes. *European journal of nutrition*, *56*(3), 1369-1373.

Costantino, S., Paneni, F., & Cosentino, F. (2015). Hyperglycemia: a bad signature on the vascular system. *Cardiovascular diagnosis and therapy*, *5*(5), 403.

Craven, P. A., Melhem, M. F., Phillips, S. L., & DeRubertis, F. R. (2001). Overexpression of Cu2+/Zn2+ SODprotects against early diabetic glomerular injury in transgenic mice. *Diabetes*, *50*(9), 2114-2125.

da Rocha Fernandes, J., Ogurtsova, K., Linnenkamp, U., Guariguata, L., Seuring, T., Zhang, P., ... & Makaroff, L. E. (2016). IDF Diabetes Atlas estimates of 2014 global health expenditures on diabetes. *Diabetes research and clinical practice*, *117*, 48-54.

Dahlin, J. L., Inglese, J., & Walters, M. A. (2015). Mitigating risk in academic preclinical drug discovery. *Nature Reviews Drug Discovery*, *14*(4), 279-294.

Daina, A., & Zoete, V. (2016). A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*, *11*(11), 1117-1121.

Daugaard, I., & Hansen, T. B. (2017). Biogenesis and function of ago-associated RNAs. *Trends in genetics*, *33*(3), 208-219.

DCCT/Edic Research Group. (2011). Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. *N Engl J Med*, *365*, 2366-2376.

Deji, N., Kume, S., Araki, S., Soumura, M., Sugimoto, T., Isshiki, K., Chin-Kanasaki, M., Sakaguchi, M., Koya, D., Haneda, M., Kashiwagi, A., and Uzu, T. (2009) Structural and functional changes in the kidneys of (HFDI) obese mice. *Am. J. Physiol. Renal Physiol.* 296, F118–F126

Denby, L., Ramdas, V., Lu, R., Conway, B. R., Grant, J. S., Dickinson, B., ... & Van Rooij, E. (2014). MicroRNA-214 antagonism protects against renal fibrosis. *Journal of the American Society of Nephrology*, *25*(1), 65-80.

Dewanjee, S., & Bhattacharjee, N. (2018). MicroRNA: A new generation therapeutic target in diabetic nephropathy. *Biochemical pharmacology*, *155*, 32-47.

Dey, N., Das, F., Mariappan, M. M., Mandal, C. C., Ghosh-Choudhury, N., Kasinath, B. S., & Choudhury, G. G. (2011). MicroRNA-21 orchestrates high glucose-induced signals to TOR complex 1, resulting in renal cell pathology in diabetes. *Journal of Biological Chemistry*, *286*(29), 25586-25603.

Di, L. (2014). The role of drug metabolizing enzymes in clearance. *Expert opinion on drug metabolism & toxicology*, *10*(3), 379-393.

DiPetrillo, K., & Gesek, F. A. (2004). Pentoxifylline ameliorates renal TNFexpression, sodium retention, and renal hypertrophy in diabetic rats. *American journal of nephrology*, *24*(3), 352-359.

DiPetrillo, K., Coutermarsh, B., & Gesek, F. A. (2003). Urinary TNFcontributes to sodium retention and renal hypertrophy during diabetes. *American Journal of Physiology-Renal Physiology*, *284*(1), F113-F121.

DiStefano, J. K., Taila, M., & Alvarez, M. L. (2013). Emerging roles for miRNAs in the development, diagnosis, and treatment of diabetic nephropathy. *Current diabetes reports*, *13*(4), 582-591.

Djordjevic, V. B. (2004). Free radicals in cell biology. *International review of cytology*, *237*, 57-91.

Dolinoy, D. C., & Jirtle, R. L. (2008). Environmental epigenomics in human health and disease. *Environmental and molecular mutagenesis*, *49*(1), 4-8.

Dolinoy, D. C., Weidman, J. R., Waterland, R. A., & Jirtle, R. L. (2006). Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environmental health perspectives*, *114*(4), 567-572.Donath, M. Y., & Shoelson, S. E. (2011). Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology*, *11*(2), 98.

Donath, M. Y. (2014). Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nature reviews Drug discovery*, *13*(6), 465.

Dong, W., Shi, H. B., Ma, H., Miao, Y. B., Liu, T. J., & Wang, W. (2010). Homoisoflavanones from Polygonatum odoratum rhizomes inhibit advanced glycation end product formation. *Archives of pharmacal research*, *33*(5), 669-674.

Dozio, E., Massaccesi, L., & Corsi Romanelli, M. M. (2021). Glycation and Glycosylation in Cardiovascular Remodeling: Focus on AGEs and O-Linked Glycosylations as Glucose-Related Pathogenetic Factors and Disease Markers. *Journal of Clinical Medicine*, *10*(20), 4792.

Druesne, N., Pagniez, A., Mayeur, C., Thomas, M., Cherbuy, C., Duée, P. H., ... & Chaumontet, C. (2004). Diallyl disulfide (DADS) increases HAc and p21 waf1/cip1 expression in human colon tumor cell lines. *Carcinogenesis*, *25*(7), 1227-1236

Duncan, B. B., Schmidt, M. I., Cousin, E., Moradi-Lakeh, M., Passos, V. M. D. A., França, E. B., ... & Mokdad, A. H. (2017). The burden of diabetes and hyperglycemia in Brazil-past and present: findings from the Global Burden of Disease Study 2015. *Diabetology & metabolic syndrome*, *9*(1), 1-12.

Egaña-Gorroño, L., López-Díez, R., Yepuri, G., Ramirez, L. S., Reverdatto, S., Gugger, P. F., ... & Schmidt, A. M. (2020). Receptor for AGEs (RAGE) and mechanisms and therapeutic opportunities in diabetes and cardiovascular disease: insights from human subjects and animal models. *Frontiers in cardiovascular medicine*, *7*, 37.

Eissa, S., Matboli, M., & Bekhet, M. M. (2016a). Clinical verification of a novel urinary microRNA panal: 133b,-342 and-30 as biomarkers for DNidentified by bioinformatics analysis. *Biomedicine & Pharmacotherapy*, *83*, 92-99.

Eissa, S., Matboli, M., Aboushahba, R., Bekhet, M. M., & Soliman, Y. (2016). Urinary exosomal microRNA panel unravels novel biomarkers for diagnosis of type 2 diabetic kidney disease. *Journal of Diabetes and its Complications*, *30*(8), 1585-1592.

Eknoyan, G., Hostetter, T., Bakris, G. L., Hebert, L., Levey, A. S., Parving, H. H., ... & Toto, R. (2003). Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK) 1. *American Journal of Kidney Diseases*, *42*(4), 617-622.

El Mesallamy, H. O., Ahmed, H. H., Bassyouni, A. A., & Ahmed, A. S. (2012). Clinical significance of inflammatory and fibrogenic cytokines in diabetic nephropathy. *Clinical biochemistry*, *45*(9), 646-650.

Esteller, M. (2008). Epigenetics in cancer. *New England Journal of Medicine*, *358*(11), 1148-1159.

Esther, G. S., & Manonmani, A. J. (2014). Effect of Eugenia Jambolana on Streptozotocin-Nicotinamide induced type-2 DNin Rats. *Int. J. Drug Dev. Res*.

Fan, S., & Zhang, X. (2009). CpG island methylation pattern in different human tissues and its correlation with gene expression. *Biochemical and biophysical research communications*, *383*(4), 421-425.

Feng, J., Wang, J., Wang, Y., Huang, X., Shao, T., Deng, X., ... & Zhao, C. (2022). Oxidative stress and lipid peroxidation: prospective associations between ferroptosis and delayed wound healing in diabetic ulcers. *Frontiers in Cell and Developmental Biology*, *10*.

Finnegan, E. F., & Pasquinelli, A. E. (2013). MicroRNA biogenesis: regulating the regulators. *Critical reviews in biochemistry and molecular biology*, *48*(1), 51-68.

for the Diabetes, T. W. T., & Control and Complications Trial/EDIC Research Group. (2002). Effect of intensive therapy on the microvascular complications of type 1 DM. *JAMA: the journal of the American Medical Association*, *287*(19), 2563.

for the Diabetes, T. W. T., & Control and Complications Trial/EDIC Research Group. (2003). Sustained effect of intensive treatment of type 1 DM on development and progression of diabetic nephropathy: the EDIC (EDIC) study. *JAMA: the journal of the American Medical Association*, *290*(16), 2159.

Forbes, J. M., Thallas, V., Thomas, M. C., Founds, H. W., Burns, W. C., Jerums, G., & Cooper, M. E. (2003). The breakdown of preexisting AGEs is associated with reduced renal fibrosis in experimental diabetes. *The FASEB Journal*, *17*(12), 1762-1764.

Fox, C. S., Matsushita, K., Woodward, M., Bilo, H. J., Chalmers, J., Heerspink, H. J. L., ... & Nelson, R. G. (2012). Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *The Lancet*, *380*(9854), 1662-1673.

Frank, R. N. (2004). Diabetic retinopathy—NEJM. *New England Journal of Medicine*, *366*, 1227-1239.

Fried, L. F., Forrest, K. Y., Ellis, D., Chang, Y., Silvers, N., & Orchard, T. J. (2001). Lipid modulation in insulin-dependent DM: effect on microvascular outcomes. *Journal of diabetes and its complications*, *15*(3), 113-119.

Fried, L. F., Orchard, T. J., & Kasiske, B. L. (2001). Effect of lipid reduction on the progression of renal disease: a meta-analysis. *Kidney international*, *59*(1), 260-269.

Friedman, R. C., Farh, K. K. H., Burge, C. B., & Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome research*, *19*(1), 92-105.

Fukami, K., Yamagishi, S. I., Ueda, S., & Okuda, S. (2008). Role of AGEs in diabetic nephropathy. *Current pharmaceutical design*, *14*(10), 946-952.

GABAlla, M. R., & Farag, Y. M. (2013). Predictors of diabetic nephropathy. *Central European Journal of Medicine*, *8*(3), 287-296.

Gambaro, G., Kinalska, I., Oksa, A., Pont'uch, P., Hertlová, M., Olsovsky, J., ... & Skrha, J. (2002). Oral sulodexide reduces albuminuria in microalbuminuric and macroalbuminuric type 1 and type 2 diabetic patients: the Di. NAS randomized trial. *Journal of the American Society of Nephrology*, *13*(6), 1615-1625.

Genuth, S., Sun, W., Cleary, P., Gao, X., Sell, D. R., Lachin, J., ... & DCCT/EDIC Research Group. (2015). Skin AGEs glucosepane and methylglyoxal hydroimidazolone are independently associated with long-term microvascular complication progression of type 1 diabetes. *Diabetes*, *64*(1), 266-278.

Gerstein, H. C., Mann, J. F., Yi, Q., Zinman, B., Dinneen, S. F., Hoogwerf, B., ... & Nawaz, S. (2001). Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *Jama*, *286*(4), 421-426.

Ghani MA, Barril C, Bedgood DR, Jr., Prenzler PD. (2017). Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. *Food Chemistry*. 230:195-207.

Ghule, A. E., Jadhav, S. S., & Bodhankar, S. L. (2012). Trigonelline ameliorates diabetic hypertensive nephropathy by suppression of oxidative stress in kidney and reduction in renal cell apoptosis and fibrosis in streptozotocin induced neonatal diabetic (nSTZ) rats. *International immunopharmacology*, *14*(4), 740-748.

Gomez, I. G., MacKenna, D. A., Johnson, B. G., Kaimal, V., Roach, A. M., Ren, S., ... & Xia, T. H. (2015). Anti–microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. *The Journal of clinical investigation*, *125*(1), 141-156.

Gooßen, K., & Gräber, S. (2012). Longer term safety of dipeptidyl peptidase-4 inhibitors in patients with type 2 DM: systematic review and meta-analysis. *Diabetes, obesity and metabolism*, *14*(12), 1061-1072.

Gorin, Y., Ricono, J. M., Wagner, B., Nam-Ho, K. I. M., Bhandari, B., Choudhury, G. G., & Abboud, H. E. (2004). Angiotensin II-induced ERK1/ERK2 activation and protein synthesis are redox-dependent in glomerular mesangial cells. *Biochemical Journal*, *381*(1), 231-239.

Govindappa, M. (2015). A review on role of plant (s) extracts and its phytochemicals for the management of diabetes. *Journal of Diabetes & Metabolism*, *6*(7), 1000565.

Grace, B. S., Clayton, P., & McDonald, S. P. (2012). Increases in renal replacement therapy in Australia and New Zealand: understanding trends in diabetic nephropathy. *Nephrology*, *17*(1), 76-84.

Grace, B. S., Clayton, P., & McDonald, S. P. (2012). Increases in renal replacement therapy in Australia and New Zealand: understanding trends in diabetic nephropathy. *Nephrology*, *17*(1), 76-84.

Grosdidier, A., Zoete, V., & Michielin, O. (2011). SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic acids research, 39(suppl_2), W270-W277.

Gu, C., Liu, S., Wang, H., & Dou, H. (2019). Role of the thioredoxin interacting protein in DNand the mechanism of regulating NOD-like receptor protein 3 inflammatory corpuscle. *International Journal of Molecular Medicine*, *43*(6), 2440-2450.

Gu, L., Hagiwara, S., Fan, Q., Tanimoto, M., Kobata, M., Yamashita, M., ... & Horikoshi, S. (2006). Role of receptor for advanced glycation end-products and signalling events in advanced glycation end-product-induced MCP-1-1 expression in differentiated mouse podocytes. *Nephrology Dialysis Transplantation*, *21*(2), 299-313.

Gujjala, S., Putakala, M., Nukala, S., Bangeppagari, M., Ramaswamy, R., Desireddy, S., 2016. Renoprotective effect of Caralluma fimbriata against (HFDI) oxidative stress in Wistar rats. J. Food Drug Anal. 24, 586–593.

Guo, C., Ding, Y., Yang, A., Geng, Y., Liu, C., Zhou, L., ... & Sun, Y. (2023). CHILKBP protects against podocyte injury by preserving ZO-1 expression. *Cellular and Molecular Life Sciences*, *80*(1), 18.

Guo, J., Li, J., Zhao, J., Yang, S., Wang, L., Cheng, G., ... & Zhao, Z. (2017). MiRNA-29c regulates the expression of inflammatory cytokines in DNby targeting tristetraprolin. *Scientific reports*, *7*(1), 2314.

Guo, L., Chen, Z., Amarnath, V., & Davies, S. S. (2012). Identification of novel bioactive aldehyde-modified phosphatidylethanolamines formed by lipid peroxidation. *Free Radical Biology and Medicine*, *53*(6), 1226-1238.

Gupta J, Juntao L, Karuturi RKM and TikooK(2012). Histone H3 lysine 4 mono methylation (H3K4me1) and H3 lysine 9 monomethylation (H3K9me1): distribution and their association in regulating gene expression under hyperglycemic/hyperinsulinemic conditions in 3T3 cells. Biochimie:94(12):2656-64.

Guttman, M., & Rinn, J. L. (2012). Modular regulatory principles of large non-coding RNAs. *Nature*, *482*(7385), 339-346.

HA, H., & LEE, H. B. (2005). ROSamplify glucose signalling in renal cells cultured under high glucose and in diabetic kidney. *Nephrology*, *10*, S7-S10.

Ha, M., & Kim, V. N. (2014). Regulation of microRNA biogenesis. *Nature reviews Molecular cell biology*, *15*(8), 509-524.

Ha, T. Y. (2011). MicroRNAs in human diseases: from cancer to cardiovascular disease. *Immune network*, *11*(3), 135-154.

Haffner, S. M. (2003). Statin therapy for the treatment of diabetic dyslipidemia. *Diabetes/metabolism research and reviews*, *19*(4), 280-287.

Hagiwara, H., Seki, T., & Ariga, T. (2004). The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. *Bioscience, biotechnology, and biochemistry*, *68*(2), 444-447.

Haigis MC, Sinclair DA (2010) Mammalian sirtuins: Biological insights and disease relevance. Annu Rev Pathol 5:253–295.

Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

Han, X., Zhang, J., Zhou, L., Wei, J., Tu, Y., Shi, Q., ... & Liang, G. (2022). Sclareol ameliorates hyperglycemia-induced renal injury through inhibiting the MAPK/NF-κB signaling pathway. *Phytotherapy Research*.

Handy, D. E., Castro, R., & Loscalzo, J. (2011). Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation*, *123*(19), 2145-2156.

Hansson, L., Zanchetti, A., Carruthers, S. G., Dahlöf, B., Elmfeldt, D., Julius, S., ... & HOT Study Group. (1998). Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. *The Lancet*, *351*(9118), 1755-1762.

Hao, H. H., Shao, Z. M., Tang, D. Q., Lu, Q., Chen, X., Yin, X. X., ... & Chen, H. (2012). Preventive effects of rutin on the development of experimental DNin rats. *Life sciences*, *91*(19-20), 959-967.

Harvey, S. J., Jarad, G., Cunningham, J., Goldberg, S., Schermer, B., Harfe, B. D., ... & Miner, J. H. (2008). Podocyte-specific deletion of dicer alters cytoskeletal dynamics and causes glomerular disease. *Journal of the American Society of Nephrology*, *19*(11), 2150-2158.

Hasegawa, K., Wakino, S., Simic, P., Sakamaki, Y., Minakuchi, H., Fujimura, K., ... & Kubota, E. (2013). Renal tubular SIRT1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. *Nature medicine*, *19*(11), 1496.

Hay, M., Thomas, D. W., Craighead, J. L., Economides, C., & Rosenthal, J. (2014). Clinical development success rates for investigational drugs. *Nature biotechnology*, *32*(1), 40-51.

Heart Protection Study Collaborative Group. (2003). MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *The Lancet*, *361*(9374), 2005-2016.

Hematyar, N., Rustad, T., Sampels, S., & Kastrup Dalsgaard, T. (2019). Relationship between lipid and protein oxidation in fish. *Aquaculture Research*, *50*(5), 1393-1403.

Hill, M., & Tran, N. (2021). miRNA interplay: Mechanisms and consequences in cancer. *Disease models & mechanisms*, *14*(4), dmm047662.

Hollenberg, P. F. (2002). Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug metabolism reviews*, *34*(1-2), 17-35.

Hou, J., & Zhao, D. (2013). MicroRNA regulation in renal pathophysiology. *International journal of molecular sciences*, *14*(7), 13078-13092.

Hsu, J. H., Jhou, B. Y., Yeh, S. H., Chen, Y. L., & Chen, C. C. (2015). Healthcare functions of Cordyceps cicadae. *Nutrition & Food Sciences*.

Huang, S. M., Strong, J. M., Zhang, L., Reynolds, K. S., Nallani, S., Temple, R., ... & Chung, S. (2008). New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. *The Journal of clinical pharmacology*, *48*(6), 662-670.

Ihnat, M. A., Thorpe, J. E., & Ceriello, A. (2007). Hypothesis: the" GM"-the new challenge of diabetes. *Clinical Diabetology*, *8*(7), 289-294.

Inoguchi, T., Sonta, T., Tsubouchi, H., Etoh, T., Kakimoto, M., Sonoda, N., ... & Utsumi, H. (2003). Protein kinase C–dependent increase in ROS(ROS) production in vascular tissues of diabetes: role of vascular NAD (P) H oxidase. *Journal of the American Society of Nephrology*, *14*(suppl 3), S227-S232.

Iova, G. M., Calniceanu, H., Popa, A., Szuhanek, C. A., Marcu, O., Ciavoi, G., & Scrobota, I. (2021). The antioxidant effect of curcumin and rutin on oxidative stress biomarkers in experimentally induced periodontitis in hyperglycemic Wistar rats. *Molecules*, *26*(5), 1332..

Ismail, A., El-Mahdy, H. A., Eldeib, M. G., & Doghish, A. S. (2022). miRNAs as cornerstones in diabetic microvascular complications. *Molecular Genetics and Metabolism*, 106978.

Ito, K., Yokota, S., Watanabe, M., Inoue, Y., Takahashi, K., Himuro, N., ... & Nakashima, H. (2021). Anemia in diabetic patients reflects severe tubulointerstitial injury and aids in clinically predicting a diagnosis of diabetic nephropathy. *Internal Medicine*, *60*(9), 1349-1357.

Jeenger, M. K., Shrivastava, S., Yerra, V. G., Naidu, V. G. M., Ramakrishna, S., & Kumar, A. (2015). Curcumin: a pleiotropic phytonutrient in diabetic complications. *Nutrition*, *31*(2), 276-282.

Jeppesen, P. L., Christensen, G. L., Schneider, M., Nossent, A. Y., Jensen, H. B., Andersen, D. C., ... & Sheikh, S. P. (2011). Angiotensin II type 1 receptor signalling regulates microRNA differentially in cardiac fibroblasts and myocytes. *British journal of pharmacology*, *164*(2), 394-404.

Jin, J., Wang, X., Zhi, X., & Meng, D. (2019). Epigenetic regulation in diabetic vascular complications. *Journal of Molecular Endocrinology*, *63*(4), R103-R115.

Jirtle, R. L., & Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature reviews genetics*, *8*(4), 253.

Jones, P. A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nature Reviews Genetics*, *13*(7), 484-492.

Jones, S., Jones, S., & Phillips, A. O. (2001). Regulation of renal proximal tubular epithelial cell hyaluronan generation: implications for diabetic nephropathy. *Kidney international*, *59*(5), 1739-1749.

Jyothilakshmi, V., Thellamudhu, G., Kumar, A., Khurana, A., Nayak, D., & Kalaiselvi, P. (2013). Preliminary investigation on ultra high diluted B. vulgaris in experimental urolithiasis. *Homeopathy*, *102*(03), 172-178.

Kadakol, A., Pandey, A., Goru, S. K., Malek, V., & Gaikwad, A. B. (2015). Insulin sensitizing and cardioprotective effects of Esculetin and Telmisartan combination by attenuating Ang II mediated vascular reactivity and cardiac fibrosis. *European journal of pharmacology*, *765*, 591-597.

Kaneko, T., Baba, N., Matsuo, M., 2003. Protection of coumarins against linoleic acid hydroperoxide-induced cytotoxicity. Chem. Biol. Interact. 142, 239–254

Kang, D., & Hamasaki, N. (2003). Mitochondrial oxidative stress and mitochondrial DNA. *Clinical chemistry and laboratory medicine*, *41*(10), 1281-1288.

Kanwar, Y. S., Sun, L., Xie, P., Liu, F. Y., & Chen, S. (2011). A glimpse of various pathogenetic mechanisms of diabetic nephropathy. *Annual Review of Pathology: Mechanisms of Disease*, *6*, 395-423.

Karpe, P. A., and Tikoo, K. (2014) Heat shock prevents insulin resistanceinduced vascular complications by augmenting angiotensin-(1–7) signaling. *Diabetes* 63, 1124–1139

Karpe, P. A., Gupta, J., Marthong, R. F., Ramarao, P., and Tikoo, K. (2012) Insulin resistance induces a segmental difference in thoracic and abdominal aorta: differential expression of AT1 and AT2 receptors. *J. Hypertens.* 30, 132–146

Kato, M., & Natarajan, R. (2014). Diabetic nephropathy—emerging epigenetic mechanisms. *Nature Reviews Nephrology*, *10*(9), 517.

Kato, M., Wang, M., Chen, Z., Bhatt, K., Oh, H. J., Lanting, L., ... & Wu, Y. (2016). An endoplasmic reticulum stress-regulated lncRNA hosting a microRNA megacluster induces early features of diabetic nephropathy. *Nature communications*, *7*, 12864..

Kato, M., Zhang, J., Wang, M., Lanting, L., Yuan, H., Rossi, J. J., & Natarajan, R. (2007). MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-β-induced collagen expression via inhibition of E-box repressors. *Proceedings of the National Academy of Sciences*, *104*(9), 3432-3437.

Keating, S. T., & El-Osta, A. (2015). Epigenetics and metabolism. *Circulation research*, *116*(4), 715-736.

Keating, S. T., van Diepen, J. A., Riksen, N. P., & El-Osta, A. (2018). Epigenetics in diabetic nephropathy, immunity and metabolism. *Diabetologia*, *61*(1), 6-20.

Kelly, D. J., Zhang, Y., Hepper, C., Gow, R. M., Jaworski, K., Kemp, B. E., ... & Gilbert, R. E. (2003). Protein kinase C β inhibition attenuates the progression of experimental DNin the presence of continued hypertension. *Diabetes*, *52*(2), 512-518.

Kelly, T. K., De Carvalho, D. D., & Jones, P. A. (2010). Epigenetic modifications as therapeutic targets. *Nature biotechnology*, *28*(10), 1069-1078.

Keppler, B. R., & Archer, T. K. (2008). Chromatin-modifying enzymes as therapeutic targets–Part 1. *Expert opinion on therapeutic targets*, *12*(10), 1301-1312.

Khan, S. U., Rahman, H., Okunrintemi, V., Riaz, H., Khan, M. S., Sattur, S., ... & Blaha, M. J. (2019). Association of lowering low-density lipoprotein cholesterol with contemporary lipid-lowering therapies and risk of DM: a systematic review and meta-analysis. *Journal of the American Heart Association*, *8*(7), e011581.

Khanra, R., Dewanjee, S., Dua, T. K., Sahu, R., Gangopadhyay, M., De Feo, V., & Zia-Ul-Haq, M. (2015). Abroma augusta L.(Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response. *Journal of translational medicine*, *13*(1), 6.

Kharb, S., Singh, V., Ghalaut, P. S., Sharma, A., & Singh, G. P. (2000). Role of oxygen free radicals in shock. *The Journal of the Association of Physicians of India*, *48*(10), 956-957.

Khella, H. W., Bakhet, M., Lichner, Z., Romaschin, A. D., Jewett, M. A., & Yousef, G. M. (2013). MicroRNAs in kidney disease: an emerging understanding. *American Journal of Kidney Diseases*, *61*(5), 798-808.

Kim, H. J., Lee, W., & Yun, J. M. (2014). Luteolin Inhibits Hyperglycemia-Induced Proinflammatory Cytokine Production and Its Epigenetic Mechanism in Human Monocytes. *Phytotherapy research*, *28*(9), 1383-1391.

Kim, J. Y., Lee, M. Y., Ji, G. E., Lee, Y. S., & Hwang, K. T. (2009). Production of γ-aminobutyric acid in black raspberry juice during fermentation by Lactobacillus brevis GABA100. *International Journal of Food Microbiology*, *130*(1), 12-16.

Kimmelstiel, P., & Wilson, C. (1936). Intercapillary lesions in the glomeruli of the kidney. *The American journal of pathology*, *12*(1), 83.

Kirchmair, J., Göller, A. H., Lang, D., Kunze, J., Testa, B., Wilson, I. D., ... & Schneider, G. (2015). Predicting drug metabolism: experiment and/or computation?. *Nature reviews Drug discovery*, *14*(6), 387-404.

Ko, G. Y. P., Yu, F., Bayless, K. J., & Ko, M. L. (2022). MicroRNA-150 (miR-150) and Diabetic Retinopathy: Is miR-150 Only a Biomarker or Does It Contribute to Disease Progression?. *International Journal of Molecular Sciences*, *23*(20), 12099.

Kobayashi, H., Nakamura, T., Miyaoka, K., Nishida, M., Funahashi, T., Yamashita, S., & Matsuzawa, Y. (2001). Visceral fat accumulation contributes to insulin resistance, small-sized low-density lipoprotein, and progression of coronary artery disease in middle-aged non-obese Japanese men. *Japanese circulation journal*, *65*(3), 193-199.

Komers, R., Oyama, T. T., Beard, D. R., Tikellis, C., Xu, B., Lotspeich, D. F., & Anderson, S. (2011). Rho kinase inhibition protects kidneys from DNwithout reducing blood pressure. *Kidney international*, *79*(4), 432-442.

Kornfeld, J. W., & Brüning, J. C. (2014). Regulation of metabolism by long, non-coding RNAs. *Frontiers in genetics*, *5*, 57.

Koroshi A. Microalbuminuria, is it so important? Hippokratia.11 (2007) 105-7.

Korpal, M., Lee, E. S., Hu, G., & Kang, Y. (2008). The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *Journal of Biological Chemistry*, *283*(22), 14910-14914.

Koteswara Rao, N. (2006). Anti-hyperglycemic and renal protective activities of Andrographis paniculata roots chloroform extract. *Iranian Journal of Pharmacology and Therapeutics*, *5*(1), 47-0.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*(4), 693-705.

Kowluru, R. A. (2003). Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. *Diabetes*, *52*(3), 818-823.

Kowluru, R. A., Kowluru, A., Mishra, M., & Kumar, B. (2015). Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. *Progress in retinal and eye research*, *48*, 40-61.

Koya, D., Hayashi, K., Kitada, M., Kashiwagi, A., Kikkawa, R., & Haneda, M. (2003). Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. *Journal of the American Society of Nephrology*, *14*(suppl 3), S250-S253.

Kumar, P., Nagarajan, A., & Uchil, P. D. (2018). Analysis of cell viability by the MTT assay. *Cold spring harbor protocols*, *2018*(6).

Kumar, S., Pamulapati, H., and Tikoo, K. (2016) Fatty acid induced METABOLIC MEMORY involves alterations in renal histone H3K36me2 and H3K27me3. *Mol. Cell. Endocrinol.* 422, 233–242

Kusirisin, W., Srichairatanakool, S., Lerttrakarnnon, P., Lailerd, N., Suttajit, M., Jaikang, C., & Chaiyasut, C. (2009). Antioxidative activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. *Medicinal Chemistry*, *5*(2), 139-147.

Lai, J. Y., Luo, J., O'Connor, C., Jing, X., Nair, V., Ju, W., ... & Zavadil, J. (2015). MicroRNA-21 in glomerular injury. *Journal of the American Society of Nephrology*, *26*(4), 805-816.

Laville, M. (2004). New strategies in anaemia management: ACORD (Anaemia CORrection in Diabetes) trial. *Acta diabetologica*, *41*(1), s18-s22.

Leask, A., & Abraham, D. J. (2004). TGF-β signaling and the fibrotic response. *The FASEB Journal*, *18*(7), 816-827.

Lee, R. C., Feinbaum, R. L., & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *cell*, *75*(5), 843-854.

Lee, W. J., Chen, Y. R., & Tseng, T. H. (2011). Quercetin induces FasL-related apoptosis, in part, through promotion of histone H3 acetylation in human leukemia HL-60 cells. *Oncology reports*, *25*(2), 583-591.

Lewis, E. J., Hunsicker, L. G., Clarke, W. R., Berl, T., Pohl, M. A., Lewis, J. B., ... & Raz, I. (2001). Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *New England Journal of Medicine*, *345*(12), 851-860.

Li, J. M., & Shah, A. M. (2003). ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy. *Journal of the American Society of Nephrology*, *14*(suppl 3), S221-S226.

Li, J., & Gobe, G. (2006). Protein kinase C activation and its role in kidney disease. *Nephrology*, *11*(5), 428-434.

Li, J., Qu, X., Ricardo, S. D., Bertram, J. F., & Nikolic-Paterson, D. J. (2010). Resveratrol inhibits renal fibrosis in the obstructed kidney: potential role in deacetylation of Smad3. *The American journal of pathology*, *177*(3), 1065-1071.

Li, L. M., Hou, D. X., Guo, Y. L., Yang, J. W., Liu, Y., Zhang, C. Y., & Zen, K. (2011). Role of microRNA-214–targeting phosphatase and tensin homolog in advanced glycation end product-induced apoptosis delay in monocytes. *The journal of immunology*, *186*(4), 2552-2560.

Li, W. L., Zheng, H. C., Bukuru, J., & De Kimpe, N. (2004). Natural medicines used in the traditional Chinese medical system for therapy of DM. *Journal of ethnopharmacology*, *92*(1), 1-21.

Li, X., Li, C., & Sun, G. (2016). HAc and its modifiers in the pathogenesis of diabetic nephropathy. *Journal of diabetes research*, *2016*.

Liang, G., Chan, M. F., Tomigahara, Y., Tsai, Y. C., Gonzales, F. A., Li, E., ... & Jones, P. A. (2002). Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Molecular and cellular biology*, *22*(2), 480-491.

Libri, V., Miesen, P., van Rij, R. P., & Buck, A. H. (2013). Regulation of microRNA biogenesis and turnover by animals and their viruses. *Cellular and Molecular Life Sciences*, *70*(19), 3525-3544.

Lindholm, L. H., Ibsen, H., Dahlöf, B., Devereux, R. B., Beevers, G., de Faire, U., ... & Lederballe-Pedersen, O. (2002). Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *The Lancet*, *359*(9311), 1004-1010.

Ling, C., & Groop, L. (2009). Epigenetics: a molecular link between environmental factors and type 2 diabetes. *Diabetes*, *58*(12), 2718-2725.

Liu, N., Guo, J.K., Pang, M., Tolbert, E., Ponnusamy, M., Gong, R., Bayliss, G., Dworkin, L.D., Yan, H., Zhuang, S., 2012. Genetic or pharmacologic blockade of EGFR inhibits renal fibrosis. J. Am. Soc. Nephrol. 23, 854–867.
Liu, N., Tolbert, E., Pang, M., Ponnusamy, M., Yan, H., Zhuang, S., 2011. Suramin inhibits renal fibrosis in chronic kidney disease. J. Am. Soc. Nephrol. 22, 1064–1075.
Liyanage, T., Ninomiya, T., Jha, V., Neal, B., Patrice, H. M., Okpechi, I., ... & Perkovic, V. (2015). Worldwide access to treatment for end-stage kidney disease: a systematic review. *The Lancet*, *385*(9981), 1975-1982.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, *4*(8), 118.

Long, D. A., Price, K. L., Herrera-Acosta, J., & Johnson, R. J. (2004). How does angiotensin II cause renal injury?. *HYPERTENSION-DALLAS-*, *43*(4), 722-723.

Long, J., Wang, Y., Wang, W., Chang, B. H., & Danesh, F. R. (2010). Identification of microRNA-93 as a novel regulator of vascular endothelial growth factor in hyperglycemic conditions. *Journal of biological chemistry*, *285*(30), 23457-23465.

Lopez-Parra, V., Mallavia, B., Egido, J., & Gomez-Guerrero, C. (2012). Immunoinflammation in diabetic nephropathy: molecular mechanisms and therapeutic options. *Diabetic Nephropathy*, 127-146.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193(1), 265-275.

Lu, H. J., Tzeng, T. F., Liou, S. S., Da Lin, S., Wu, M. C., & Liu, I. M. (2014). Ruscogenin ameliorates DNby its anti-inflammatory and anti-fibrotic effects in streptozotocin-induced diabetic rat. *BMC complementary and alternative medicine*, *14*(1), 110.

Lu, J., Huang, Y., Zhang, X., Xu, Y., & Nie, S. (2021). Noncoding RNAs involved in DNAMeand histone methylation, and acetylation in diabetic vascular complications. *Pharmacological Research*, *170*, 105520.

Lu, X., Wang, L., Yu, C., Yu, D., & Yu, G. (2015). HAc modifiers in the pathogenesis of Alzheimer's disease. *Frontiers in cellular neuroscience*, *9*, 226.

MacIsaac, R. J., Tsalamandris, C., Panagiotopoulos, S., Smith, T. J., McNeil, K. J., & Jerums, G. (2004). Nonalbuminuric renal insufficiency in type 2 diabetes. *Diabetes care*, *27*(1), 195-200.

Magee, C., Grieve, D. J., Watson, C. J., & Brazil, D. P. (2017). Diabetic nephropathy: a tangled web to unweave. *Cardiovascular drugs and therapy*, *31*(5), 579-592.

Maitra, A. Endocrine system. In kumar V. Fausto N, Abbas AK (eds). Robbins and Cortan Pathologic basis of disease 2005.

Mani, U. V., Mani, I., Biswas, M., & Kumar, S. N. (2011). An open-label study on the effect of flax seed powder (Linum usitatissimum) supplementation in the management of DM. *Journal of dietary supplements*, *8*(3), 257-265.

Mannervik, B., Helena Danielson, U., & Ketterer, B. (1988). Glutathione transferases—structure and catalytic activit. *Critical Reviews in Biochemistry*, *23*(3), 283-337.

Mariño-Ramírez, L., Kann, M. G., Shoemaker, B. A., & Landsman, D. (2005). Histone structure and nucleosome stability. *Expert review of proteomics*, *2*(5), 719-729.

Martin, Y. C., Kofron, J. L., & Traphagen, L. M. (2002). Do structurally similar molecules have similar biological activity? Journal of medicinal chemistry, 45(19), 4350-4358.

Martinez-Jimenez, C. P., & Sandoval, J. (2015). Epigenetic crosstalk: a molecular language in human metabolic disorders. *Frontiers in Bioscience-Scholar*, *7*(1), 46-57.

Mason, R. M., & Wahab, N. A. (2003). ECM metabolism in diabetic nephropathy. *Journal of the American Society of Nephrology*, *14*(5), 1358-1373.

Mathiyalagan, P., Keating, S. T., Du, X. J., & El-Osta, A. (2014). Chromatin modifications remodel cardiac gene expression. *Cardiovascular Research*, *103*(1), 7-16.

Matteucci, E., & Giampietro, O. (2000). Oxidative stress in families of type 1 diabetic patients. *Diabetes care*, *23*(8), 1182-1186.

Meeran, S. M., Patel, S. N., & Tollefsbol, T. O. (2010). Sulforaphane causes epigenetic repression of hTERT expression in human breast cancer cell lines. *PloS one*, *5*(7), e11457.

Mehta, D., Rahman, A., & Malik, A. B. (2001). Protein kinase C-α signals rho-guanine nucleotide dissociation inhibitor phosphorylation and rho activation and regulates the endothelial cell barrier function. *Journal of Biological Chemistry*, *276*(25), 22614-22620.

Melo, C. A., & Melo, S. A. (2014). Biogenesis and physiology of microRNAs. In *Non-coding RNAs and Cancer* (pp. 5-24). Springer, New York, NY.

Meng, X. M., Nikolic-Paterson, D. J., & Lan, H. Y. (2016). TGF-β: the master regulator of fibrosis. *Nature Reviews Nephrology*, *12*(6), 325.

Miao, F., Chen, Z., Genuth, S., Paterson, A., Zhang, L., Wu, X., ... & DCCT/EDIC Research Group. (2014). Evaluating the role of epigenetic histone modifications in the METABOLIC MEMORY of type 1 diabetes. *Diabetes*, *63*(5), 1748-1762.

Miao, F., Gonzalo, I. G., Lanting, L., & Natarajan, R. (2004). In vivo chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. *Journal of Biological Chemistry*, *279*(17), 18091-18097.

Michan S, Sinclair D (2007) Sirtuins in mammals: Insights into their biological function. Biochem J 404(1):1–13.

Mishra, M., Zhong, Q., & Kowluru, R. A. (2014). Epigenetic modifications of Keap1 regulate its interaction with the protective factor Nrf2 in the development of diabetic retinopathy. *Investigative ophthalmology & visual science*, *55*(11), 7256-7265.

Mitchell, N. S., Scialla, J. J., & Yancy Jr, W. S. (2020). Are low-carbohydrate diets safe in diabetic and nondiabetic chronic kidney disease?. *Annals of the New York Academy of Sciences*, *1461*(1), 25-36.

Mogensen, C. E. (2003). Microalbuminuria and hypertension with focus on type 1 and type 2 diabetes. *Journal of internal medicine*, *254*(1), 45-66.

Molitch, M. E., DeFronzo, R. A., Franz, M. J., & Keane, W. F. (2004). Nephropathy in diabetes. *Diabetes care*, *27*, S79.

Montanari, F., & Ecker, G. F. (2015). Prediction of drug–ABC-transporter interaction—Recent advances and future challenges. *Advanced drug delivery reviews*, *86*, 17-26.

Montigny, P., Degroof, A., Brusa, D., Houssiau, F., & Lauwerys, B. (2020). P104 Correlation between interstitial CD8+ T cell infiltration and fibrotic processes in a mouse model of lupus nephritis.

Moon, J. Y., Jeong, K. H., Lee, T. W., Ihm, C. G., Lim, S. J., & Lee, S. H. (2012). Aberrant recruitment and activation of T cells in diabetic nephropathy. *American journal of nephrology*, *35*(2), 164-174.

Moriwaki, Y., Inokuchi, T., Yamamoto, A., Ka, T., Tsutsumi, Z., Takahashi, S., & Yamamoto, T. (2007). Effect of TNF-α inhibition on urinary albumin excretion in experimental diabetic rats. *Acta diabetologica*, *44*(4), 215-218.

Morrish, N. J., Wang, S. L., Stevens, L. K., Fuller, J. H., Keen, H., & WHO Multinational Study Group. (2001). Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia*, *44*, S14-S21.

Mu, J., Pang, Q., Guo, Y. H., Chen, J. G., Zeng, W., Huang, Y. J., ... & Feng, B. (2013). Functional implications of microRNA-215 in TGF-β1-induced phenotypic transition of mesangial cells by targeting CTNNBIP1. *PloS one*, *8*(3), e58622.

Nagai, Y., Matoba, K., Takeda, Y., Yako, H., Akamine, T., Sekiguchi, K., ... & Nishimura, R. (2022). Rho-associated, coiled-coil–containing protein kinase 1 regulates development of DKDvia modulation of fatty acid metabolism. *Kidney International*.

Nakano, T., Mizumasa, T., Kuroki, Y., Eriguchi, M., Yoshida, H., Taniguchi, M., ... & Kitazono, T. (2020). AGEs are associated with immature angiogenesis and peritoneal dysfunction in patients on peritoneal dialysis. *Peritoneal Dialysis International*, *40*(1), 67-75.

Nassiri-Asl, M., & Hosseinzadeh, H. (2009). Review of the pharmacological effects of Vitis vinifera (Grape) and its bioactive compounds. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *23*(9), 1197-1204.

Nathan, D. M. (2005). DCCT/EDIC (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med*, *353*, 2643-2653.

Navarro-Gonzalez, J. F., & Mora-Fernandez, C. (2008). The role of inflammatory cytokines in diabetic nephropathy. *Journal of the American Society of Nephrology*, *19*(3), 433-442.

Navarro-González, J. F., Jarque, A., Muros, M., Mora, C., & García, J. (2009). Tumor necrosis factor-α as a therapeutic target for diabetic nephropathy. *Cytokine & growth factor reviews*, *20*(2), 165-173.

Negre-Salvayre, A., Salvayre, R., Augé, N., Pamplona, R., & Portero-Otin, M. (2009). Hyperglycemia and glycation in diabetic complications. *Antioxidants & redox signaling*, *11*(12), 3071-3109.

Nenadis, N., Tsimidou, M., 2002. Observations of the estimation of scavenging activity of phenolic compounds using rapid 1,1-Diphenyl-2-picrylhudrazyl (DPPH) Tests. JAOCS 79, 1191–1195.

Neugebauer RC, Sippl W, Jung M (2008) Inhibitors of NAD+ dependent HDAC (sirtuins). Curr Pharm Des 14(6):562–573.

Noeman, S. A., Hamooda, H. E., & Baalash, A. A. (2011). Biochemical study of oxidative stress markers in the liver, kidney and heart of HFD-induced obesity in rats. *Diabetology & metabolic syndrome*, *3*(1)

Ojha, S., Alkaabi, J., Amir, N., Sheikh, A., Agil, A., Fahim, M. A., & Adem, A. (2014). Withania coagulans fruit extract reduces oxidative stress and inflammation in kidneys of streptozotocin-induced diabetic rats. *Oxidative medicine and cellular longevity*, *2014*.

Okada, T., Sugishita, T., Murakami, T., Murai, H., Saikusa, T., Horino, T., ... & Takahashi, T. (2000). Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. *Nippon Shokuhin Kagaku Kogaku Kaishi= Journal of the Japanese Society for Food Science and Technology*, *47*(8), 596-603.

Okano, M., Bell, D. W., Haber, D. A., & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, *99*(3), 247-257.

Okhuarobo, A., Falodun, J. E., Erharuyi, O., Imieje, V., Falodun, A., & Langer, P. (2014). Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pacific journal of tropical disease*, *4*(3), 213-222.

Ooi, S. K., & Bestor, T. H. (2008). The colorful history of active DNA demethylation. *Cell*, *133*(7), 1145-1148.

Ottaviani, G., Gosling, D. J., Patissier, C., Rodde, S., Zhou, L., & Faller, B. (2010). What is modulating solubility in simulated intestinal fluids?. *European journal of pharmaceutical sciences*, *41*(3-4), 452-457

Pacholec, M., Bleasdale, J.E., Chrunyk, B., Cunningham, D., Flynn, D., Garofalo, R.S., Griffith, D., Griffor, M., Loulakis, P., Pabst, B., 2010. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J. Biol. Chem. 285, 8340–8351.

Pandey, K. B., Mishra, N., & Rizvi, S. I. (2010). Protein oxidation biomarkers in plasma of type 2 diabetic patients. *Clinical biochemistry*, *43*(4-5), 508-511.

Paneni, F., Beckman, J. A., Creager, M. A., & Cosentino, F. (2013). Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *European heart journal*, *34*(31), 2436-2443.

Pang, M., Kothapally, J., Mao, H., Tolbert, E., Ponnusamy, M., Chin, Y.E., Zhuang, S., 2009. Inhibition of histone deacetylase activity attenuates renal fibroblast activation and interstitial fibrosis in obstructive nephropathy. Am. J. Physiol. Ren. Physiol. 297, F996–F1005.

Pang, M., Li, Y., Gu, W., Sun, Z., Wang, Z., & Li, L. (2021). Recent advances in epigenetics of macrovascular complications in DM. *Heart, Lung and Circulation*, *30*(2), 186-196.

Park, K. B., & Oh, S. H. (2007). Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. *Bioresource Technology*, *98*(8), 1675-1679.

Park, S. M., Gaur, A. B., Lengyel, E., & Peter, M. E. (2008). The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes & development*, *22*(7), 894-907.

Parving, H. H., Oxenbøll, B., Svendsen, P. A., Christiansen, J. S., & Andersen, A. R. (1982). Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. *European Journal of Endocrinology*, *100*(4), 550-555.

Parving, H. H., Persson, F., Lewis, J. B., Lewis, E. J., & Hollenberg, N. K. (2008). Aliskiren combined with losartan in type 2 diabetes and nephropathy. *New England Journal of Medicine*, *358*(23), 2433-2446.

Patel, H., Chen, J., Das, K. C., & Kavdia, M. (2013). Hyperglycemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. *Cardiovascular diabetology*, *12*(1), 1-14.

Patel, V. J., Joharapurkar, A. A., Kshirsagar, S. G., Sutariya, B. K., Patel, M. S., Patel, H. M., ... & Jain, M. R. (2018). Coagonist of glucagon-like peptide-1 and glucagon receptors ameliorates kidney injury in murine models of obesity and DM. *World journal of diabetes*, *9*(6), 80.

Paul, P., Chakraborty, A., Sarkar, D., Langthasa, M., Rahman, M., Bari, M., ... & Chakraborty, S. (2018). Interplay between miRNAs and human diseases. *Journal of cellular physiology*, *233*(3), 2007-2018.

Peng, A., Gu, Y., & Lin, S. Y. (2005). Herbal treatment for renal diseases. *Ann Acad Med Singapore*, *34*(1), 44-51.

Peng, Y., Lin, H., Tian, S., Liu, S., Li, J., Lv, X., ... & Shao, Z. (2021). Glucagon-like peptide-1 receptor activation maintains extracellular matrix integrity by inhibiting the activity of mitogen-activated protein kinases and activator protein-1. *Free Radical Biology and Medicine*, *177*, 247-259.

Pirola, L., Balcerczyk, A., Okabe, J., & El-Osta, A. (2010). Epigenetic phenomena linked to diabetic complications. *Nature Reviews Endocrinology*, *6*(12), 665.

Pirola, L., Balcerczyk, A., Tothill, R. W., Haviv, I., Kaspi, A., Lunke, S., ... & Beresford-Smith, B. (2011). Genome-wide analysis distinguishes hyperglycemia regulated epigenetic signatures of primary vascular cells. *Genome research*, *21*(10), 1601-1615.

Pollack, R. M., Donath, M. Y., LeRoith, D., & Leibowitz, G. (2016). Anti-inflammatory agents in the treatment of diabetes and its vascular complications. *Diabetes Care*, *39*(Supplement 2), S244-S252.
Ponnusamy, M., Zhou, X., Yan, Y., Tang, J., Tolbert, E., Zhao, T.C., Gong, R., Zhuang, S.,2014. Blocking sirtuin 1 and 2 inhibits renal interstitial fibroblast activation and attenuates renal interstitial fibrosis in obstructive nephropathy. J. Pharmacol. Exp. Ther. 350, 243–256.
Ponnusamy, M., Zhuang, M. A., Zhou, X., Tolbert, E., Bayliss, G., Zhao, T. C., & Zhuang, S. (2015). Activation of sirtuin-1 promotes renal fibroblast activation and aggravates renal fibrogenesis. *Journal of Pharmacology and Experimental Therapeutics*, *354*(2), 142-151.
Portela, A., & Esteller, M. (2010). Epigenetic modifications and human disease. *Nature biotechnology*, *28*(10), 1057-1068.

Rafieian-Kopaie, M and Nasri, H. (2012). Silymarin and diabetic nephropathy. Journal of Renal Injury Prevention.

Rajab, D. H., & Mohammad, W. M. (2022). Relationship Between Protein Kinase C and DNPatients in Al Anbar City. *Pakistan Journal of Medical & Health Sciences*, *16*(06), 632-632.

Ramakrishnan, V. (1997). Histone structure and the organization of the nucleosome. *Annual review of biophysics and biomolecular structure*, *26*(1), 83-112.

Raman, M., Chen, W., & Cobb, M. H. (2007). Differential regulation and properties of MAPKs. *Oncogene*, *26*(22), 3100.

Ramassone, A., Pagotto, S., Veronese, A., & Visone, R. (2018). Epigenetics and microRNAs in cancer. *International journal of molecular sciences*, *19*(2), 459.

Reddy, M. A., & Natarajan, R. (2011). Epigenetic mechanisms in diabetic vascular complications. *Cardiovascular research*, *90*(3), 421-429.

Reddy, M. A., Jin, W., Villeneuve, L., Wang, M., Lanting, L., Todorov, I., ... & Natarajan, R. (2012). Pro-inflammatory role of microrna-200 in vascular smooth muscle cells from diabetic mice. *Arteriosclerosis, thrombosis, and vascular biology*, *32*(3), 721-729.

Reddy, M. A., Park, J. T., & Natarajan, R. (2013, July). Epigenetic modifications in the pathogenesis of diabetic nephropathy. In *Seminars in nephrology* (Vol. 33, No. 4, pp. 341-353). WB Saunders.

Reddy, M. A., Sahar, S., Villeneuve, L. M., Lanting, L., & Natarajan, R. (2009). Role of Src tyrosine kinase in the atherogenic effects of the 12/15-lipoxygenase pathway in vascular smooth muscle cells. *Arteriosclerosis, thrombosis, and vascular biology*, *29*(3), 387-393.

Riancho, J., Del Real, A., & Riancho, J. A. (2016). How to interpret epigenetic association studies: a guide for clinicians. *BoneKEy Reports*, *5*.

Ripsin, C. M., Kang, H., & Urban, R. J. (2009). Management of blood glucose in type 2 DM. *American family physician*, *79*(1), 29-36.

Ritchie, T. J., Macdonald, S. J., Peace, S., Pickett, S. D., & Luscombe, C. N. (2013). Increasing small molecule drug developability in sub-optimal chemical space. *MedChemComm*, *4*(4), 673-680.

Roberts, E., & Eidelberg, E. (1960). Metabolic and neurophysiological roles of γ-aminobutyric acid. In *International review of neurobiology* (Vol. 2, pp. 279-332). Academic Press.

Roberts, E., & Frankel, S. (1950). γ-aminobutyric acid in brain: its formation from glutamic acid. *Journal of Biological Chemistry*, *187*, 55-63.

Rorsman, P., Berggren, P. O., Bokvist, K., Ericson, H., Möhler, H., Östenson, C. G., & Smith, P. A. (1989). Glucose-inhibition of glucagon secretion involves activation of GABA A-receptor chloride channels. *Nature*, *341*(6239), 233-236.

Roth, S. Y., Denu, J. M., & Allis, C. D. (2001). Histone acetyltransferases. *Annual review of biochemistry*, *70*(1), 81-120.

Rupaimoole, R., & Slack, F. J. (2017). MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nature reviews Drug discovery*, *16*(3), 203.

Sabu, M. C., & Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of ethnopharmacology*, *81*(2), 155-160.

Sacks, D. B., Bruns, D. E., Goldstein, D. E., Maclaren, N. K., McDonald, J. M., & Parrott, M. (2002). Guidelines and recommendations for laboratory analysis in the diagnosis and management of DM. *Clinical chemistry*, *48*(3), 436-472.

Sailaja, Y. R., Baskar, R., & Saralakumari, D. (2003). The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. *Free Radical Biology and Medicine*, *35*(2), 133-139.

Salau, V. F., Erukainure, O. L., Olofinsan, K. A., Ijomone, O. M., Msomi, N. Z., & Islam, M. (2021). Vanillin modulates activities linked to dysmetabolism in psoas muscle of diabetic rats. *Scientific reports*, *11*(1), 1-17.

Salvemini D, Cuzzocrea S. Therapeutic potential of SODmimetics as therapeutic agents in critical care medicine. Crit. Care. Med, 2003, 31: 29-38. 41.

Sanchez, A. P., & Sharma, K. (2009). Transcription factors in the pathogenesis of diabetic nephropathy. *Expert reviews in molecular medicine*, *11*.

Sapienza, C., Lee, J., Powell, J., Erinle, O., Yafai, F., Reichert, J., ... & Madaio, M. (2011). DNAMe profiling identifies epigenetic differences between diabetes patients with ESRD and diabetes patients without nephropathy. *Epigenetics*, *6*(1), 20-28.

Saravanan, S., Pari, L., 2016. Protective effect of thymol on HFD induced DNin C57BL/6J mice. Chem. Biol. Interact. 245, 1–11.

Sarras Jr, M. P., Leontovich, A. A., & Intine, R. V. (2015). Use of zebrafish as a model to investigate the role of epigenetics in propagating the secondary complications observed in DM. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *178*, 3-7.

Sauve AA, Wolberger C, Schramm VL, Boeke JD (2006) The biochemistry of sirtuins. Annu Rev Biochem 75:435–465.

Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug solubility: importance and enhancement techniques. *ISRN pharmaceutics*, *2012*.

Sawai, Y., Yamaguchi, Y., Miyama, D., & Yoshitomi, H. (2001). Cycling treatment of anaerobic and aerobic incubation increases the content of γ-aminobutyric acid in tea shoots. *Amino acids*, *20*(3), 331-334.

Schiffer, M., Von Gersdorff, G., Bitzer, M., Susztak, K., & Böttinger, E. P. (2000). Smad proteins and transforming growth factor-β signaling. *Kidney International*, *58*, S45-S52.

Schwartz, S., Fonseca, V., Berner, B., Cramer, M., Chiang, Y. K., & Lewin, A. (2006). Efficacy, tolerability, and safety of a novel once-daily extended-release metformin in patients with type 2 diabetes. *Diabetes Care*, *29*(4), 759-764.

Sedeek, M., Montezano, A. C., Hebert, R. L., Gray, S. P., Di Marco, E., Jha, J. C., ... & Touyz, R. M. (2012). Oxidative stress, Nox isoforms and complications of diabetes—potential targets for novel therapies. *Journal of cardiovascular translational research*, *5*(4), 509-518.

Seegmiller, J. C., Eckfeldt, J. H., & Lieske, J. C. (2018). Challenges in measuring glomerular filtration rate: a clinical laboratory perspective. *Advances in chronic kidney disease*, *25*(1), 84-92.

Selvaraj, G., Kaliamurthi, S., & Thirugnanasambandam, R. (2014). Molecular docking studies on potential PPAR-γ agonist from Rhizophora apiculata. ||| Bangladesh Journal of Pharmacology|||, 9(3), 298-302.
Sequeira-Lopez, M. L. S., Weatherford, E. T., Borges, G. R., Monteagudo, M. C., Pentz, E. S., Harfe, B. D., ... & Gomez, R. A. (2010). The microRNA-processing enzyme dicer maintains juxtaglomerular cells. *Journal of the American Society of Nephrology*, *21*(3), 460-467.

Seto, E., & Yoshida, M. (2014). Erasers of HAc: the histone deacetylase enzymes. *Cold Spring Harbor perspectives in biology*, *6*(4), a018713.

Shafi, S., Tabassum, N., & Ahmad, F. (2012). DNand herbal medicines. *International Journal of Phytopharmacology*, *3*(1), 10-17.

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. Food chemistry, 113(4), 1202-1205

Sharom, F. J. (2008). ABC multidrug transporters: structure, function and role in chemoresistance.

Shukla, K., Dikshit, P., Tyagi, M. K., Shukla, R., & Gambhir, J. K. (2012). Ameliorative effect of Withaniacoagulans on dyslipidemia and oxidative stress in nicotinamide–streptozotocin induced DM. *Food and Chemical Toxicology*, *50*(10), 3595-3599.

Shvedunova, M., & Akhtar, A. (2022). Modulation of cellular processes by histone and non-histone protein acetylation. *Nature reviews Molecular cell biology*, *23*(5), 329-349.

Spiteller, G. (2003). Are LPOprocesses induced by changes in the cell wall structure and how are these processes connected with diseases?. *Medical hypotheses*, *60*(1), 69-83.
Squibb, B. M. (2002). Glucophage® [Metformin Hydrochloride Tablets] and Glucophage® XR [Metformin Hydrochloride Extended-Release Tablets] Product Information. *Princeton, NJ: Bristol-Myers Squibb*.

Steward, F. C. (1949). γ-Aminobutyric acid: a constituent of the potato tuber?. *Science*, *110*, 439-440.

Straussman, R., Nejman, D., Roberts, D., Steinfeld, I., Blum, B., Benvenisty, N., ... & Cedar, H. (2009). Developmental programming of CpG island methylation profiles in the human genome. *Nature structural & molecular biology*, *16*(5), 564.

Sun J, Wang Y, Cui W, Lou Y, Sun G, Zhang D, and Miao L (2017). Role of Epigenetic Histone Modifications in DKDInvolving Renal Fibrosis. Journal of Diabetes Research.

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., ... & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, *183*, 109119.

Sun, X. Y., Qin, H. J., Zhang, Z., Xu, Y., Yang, X. C., Zhao, D. M., ... & Sun, L. K. (2016). Valproate attenuates DNthrough inhibition of endoplasmic reticulum stress-induced apoptosis. *Molecular medicine reports*, *13*(1), 661-668.

Sutariya, B., & Saraf, M. (2017). Betanin, isolated from fruits of Opuntia elatior Mill attenuates renal fibrosis in diabetic rats through regulating oxidative stress and TGF-β pathway. *Journal of ethnopharmacology*, *198*, 432-443.

Swan, E. J., Maxwell, A. P., & McKnight, A. J. (2015). Distinct methylation patterns in genes that affect mitochondrial function are associated with kidney disease in blood-derived DNA from individuals with Type 1 diabetes. *Diabetic Medicine*, *32*(8), 1110-1115.

Szakacs, G., Varadi, A., Özvegy-Laczka, C., & Sarkadi, B. (2008). The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME–Tox). *Drug discovery today*, *13*(9-10), 379-393.

Takino, J. I., Sato, T., Kanetaka, T., Okihara, K., Nagamine, K., Takeuchi, M., & Hori, T. (2021). RasGRP2 inhibits glyceraldehyde-derived toxic advanced glycation end-products from inducing permeability in vascular endothelial cells. *Scientific reports*, *11*(1), 1-10.

Tallapragada, D. S., Karpe, P. A., and Tikoo, K. (2015) Long-lasting partnership between insulin resistance and endothelial dysfunction: role of METABOLIC MEMORY. *Br. J. Pharmacol.* 172, 4012–4023

Tana, C., Giamberardino, M. A., & Cipollone, F. (2017). microRNA profiling in atherosclerosis, diabetes, and migraine. *Annals of medicine*, *49*(2), 93-105.

Tang, C., Wang, M., Liu, J., Zhang, C., Li, L., Wu, Y., ... & Yuan, X. (2022). A Cyclopentanone Compound Attenuates the Over-Accumulation of ECM and Fibrosis in DNvia Downregulating the TGF-β/p38MAPK Axis. *Biomedicines*, *10*(12), 3270.

Tang, D., Yu, Y., Zheng, X., Wu, J., Li, Y., Wu, X., ... & Yin, X. (2014). Comparative investigation of in vitro biotransformation of 14 components in Ginkgo biloba extract in normal, diabetes and DNrat intestinal bacteria matrix. *Journal of pharmaceutical and biomedical analysis*, *100*, 1-10.

Testa, B., & Kraemer, S. D. (2007). The Biochemistry of Drug Metabolism–An Introduction-Testa-2007-Chemistry & Biodiversity-Wiley Online Library. *Chem. Biodivers*, *4*(3).

Testa, R., Bonfigli, A. R., Prattichizzo, F., La Sala, L., De Nigris, V., & Ceriello, A. (2017). The "METABOLIC MEMORY" theory and the early treatment of hyperglycemia in prevention of diabetic complications. *Nutrients*, *9*(5), 437.

Tian, Z., Greene, A. S., Pietrusz, J. L., Matus, I. R., & Liang, M. (2008). MicroRNA–target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. *Genome research*, *18*(3), 404-411.

Tikoo K, Bhatt DK, Gaikwad AB, Sharma V, Kabra DG. 2007a. Differential effects of tannic acid on cisplatin induced nephrotoxicity in rats. FEBS Lett 581:2027–2035.

Tikoo K, Tripathi DN, Kabra DG, Sharma V, Gaikwad AB. 2007b. Intermittent fasting prevents the progression of type I diabetic nephropathy in rats and changes the expression of Sir2 and p53. FEBS Lett 581:1071–1078.

Tikoo, K., Sharma, E., Amara, V. R., Pamulapati, H., & Dhawale, V. S. (2016). Metformin Improves METABOLIC MEMORY in HFD (HFD)-induced Renal Dysfunction*♦. *Journal of biological chemistry*, *291*(42), 21848-21856.

Torres, R., Faini, F., Modak, B., Urbina, F., Labbe, C., Guerrero, J., 2006. Antioxidant activity of coumarins and flavonols from the resinous exudate of Haplopappus multifolius. Phytochemistry 67, 984–987

Toth, P. P. (2005). The "good cholesterol" high-density lipoprotein. *Circulation*, *111*(5), e89-e91.

Traka, M., Gasper, A. V., Smith, J. A., Hawkey, C. J., Bao, Y., & Mithen, R. F. (2005). Transcriptome analysis of human colon Caco-2 cells exposed to sulforaphane. *The Journal of nutrition*, *135*(8), 1865-1872.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, 31(2), 455-461.

Trujillo, H., Caravaca-Fontán, F., Caro, J., Morales, E., & Praga, M. (2021). The forgotten antiproteinuric properties of diuretics. *American Journal of Nephrology*, *52*(6), 435-449.

Tsikas, D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical biochemistry*, *524*, 13-30.

Tsuruhisa, S., Matsui, T., Koga, Y., Sotokawauchi, A., Yagi, M., & Yamagishi, S. I. (2021). Pimetabolic memoryent epithelium-derived factor inhibits advanced glycation end product-induced proliferation, VEGF and MMP-9 expression in breast cancer cells via interaction with laminin receptor. *Oncology Letters*, *22*(2), 1-9.

Ueda, Y., Doi, T., Nagatomo, K., Tokumaru, J., Takaki, M., & Willmore, L. J. (2007). Effect of levetiracetam on molecular regulation of hippocampal glutamate and GABA transporters in rats with chronic seizures induced by amygdalar FeCl3 injection. *Brain research*, *1151*, 55-61.

UK Prospective Diabetes Study (UKPDS) Group. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The lancet*, *352*(9131), 837-853.

Utimura, R., Fujihara, C. K., Mattar, A. L., Malheiros, D. M. A. C., de Lourdes Noronha, I., & Zatz, R. (2003). Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. *Kidney international*, *63*(1), 209-216.

Valero, M. A., Vidal, A., Burgos, R., Calvo, F. L., Martínez, C., Luengo, L. M., & Cuerda, C. (2011). Meta-analysis on the role of lycopene in type 2 DM.

van Waterschoot, R. A., & Schinkel, A. H. (2011). A critical analysis of the interplay between cytochrome P450 3A and P-glycoprotein: recent insights from knockout and transgenic mice. *Pharmacological reviews*, *63*(2), 390-410.

Viberti, G., & Wheeldon, N. M. (2002). Microalbuminuria reduction with valsartan in patients with type 2 DM: a blood pressure–independent effect. *Circulation*, *106*(6), 672-678.

Villeneuve, L. M., Reddy, M. A., & Natarajan, R. (2011). Epigenetics: deciphering its role in diabetes and its chronic complications. *Clinical and Experimental Pharmacology and Physiology*, *38*(7), 451-459.

Villota-Salazar, N. A., Mendoza-Mendoza, A., & González-Prieto, J. M. (2016). Epigenetics: from the past to the present. *Frontiers in Life Science*, *9*(4), 347-370.

Vistica VT, Skehan P, Scudiero D, Monks A, Pittman A, Boyd MR. 1991. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. Cancer Res. 51(10):2515-20.

Viswanathan, V., Snehalatha, C., Kumutha, R., Jayaraman, M., Ramachandran, A., 2004. Serum albumin levels in different stages of type 2 DNpatients. Indian. J. Nephrol. 14, 89–92.

Waddington, C. H. (1942). The epigenotype. *Endeavour*, *1*, 18-20.

Wake, N. (2000). Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes care*, *23*, 21-29.

Wang, J., Wu, Z., Li, D., Li, N., Dindot, S. V., Satterfield, M. C., ... & Wu, G. (2012). Nutrition, epigenetics, and metabolic syndrome. *Antioxidants & redox signaling*, *17*(2), 282-301.

Wang, X. X., Jiang, T., & Levi, M. (2010). Nuclear hormone receptors in diabetic nephropathy. *Nature Reviews Nephrology*, *6*(6), 342-351.

Wang, X., Huang, S., Xin, X., Ren, Y., WENG, G., & Wang, P. (2019). The antitumor activity of UMB in human renal cell carcinoma via regulation of the p110γ catalytic subunit of PI3Kγ. *Acta Pharmaceutica*, *69*(1), 111-119.

Wang, Y., Miao, X., Liu, Y., Li, F., Liu, Q., Sun, J., & Cai, L. (2014). Dysregulation of histone acetyltransferases and deacetylases in cardiovascular diseases. *Oxidative medicine and cellular longevity*, *2014*.

Wei, J., Zhang, Y., Luo, Y., Wang, Z., Bi, S., Song, D., ... & Yuan, L. (2014). Aldose reductase regulates miR-200a-3p/141-3p to coordinate Keap1–Nrf2, Tgfβ1/2, and Zeb1/2 signaling in renal mesangial cells and the renal cortex of diabetic mice. *Free Radical Biology and Medicine*, *67*, 91-102.

Weng, J., Chen, Z., Li, J., He, Q., Chen, D., Yang, L., ... & Guo, X. (2021). AGEs induce endothelial hyperpermeability via β-catenin phosphorylation and subsequent up-regulation of ADAM10. *Journal of Cellular and Molecular Medicine*, *25*(16), 7746-7759.

Whelton, P. K., Carey, R. M., Aronow, W. S., Casey, D. E., Collins, K. J., Dennison Himmelfarb, C., ... & Wright, J. T. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journal of the American College of Cardiology*, *71*(19), e127-e248.

White, K. E., & Bilous, R. W. (2000). Type 2 diabetic patients with nephropathy show structural—functional relationships that are similar to type 1 disease. *Journal of the American Society of Nephrology*, *11*(9), 1667-1673.

Whitelaw, N. C., & Whitelaw, E. (2006). How lifetimes shape epigenotype within and across generations. *Human molecular genetics*, *15*(suppl_2), R131-R137.

Williams, K. T., & Schalinske, K. L. (2012). Tissue-specific alterations of methyl group metabolism with DNA hypermethylation in the Zucker (type 2) diabetic fatty rat. *Diabetes/metabolism research and reviews*, *28*(2), 123-131.

Wolf, C. R., Smith, G., & Smith, R. L. (2000). Pharmacogenetics. *Bmj*, *320*(7240), 987-990.

Wolf, G. (2006). Renal injury due to renin–angiotensin–aldosterone system activation of the transforming growth factor-β pathway. *Kidney international*, *70*(11), 1914-1919.

World Health Organization. (2017). *Global diffusion of eHealth: making universal health coverage achievable: report of the third global survey on eHealth*. World Health Organization.

Woroniecka, K. I., Park, A. S. D., Mohtat, D., Thomas, D. B., Pullman, J. M., & Susztak, K. (2011). Transcriptome analysis of human diabetic kidney disease. *Diabetes*, *60*(9), 2354-2369.

Wu, C. C., Hung, C. N., Shin, Y. C., Wang, C. J., & Huang, H. P. (2016). Myrciaria cauliflora extracts attenuate DNinvolving the Ras signaling pathway in streptozotocin/nicotinamide mice on a HFD. *journal of food and drug analysis*, *24*(1), 136-146.

Wu, H., Kong, L., Zhou, S., Cui, W., Xu, F., Luo, M., ... & Miao, L. (2014). The role of microRNAs in diabetic nephropathy. *Journal of diabetes research*, *2014*.

Wu, L., Wang, Q., Guo, F., Ma, X., Ji, H., Liu, F., ... & Qin, G. (2016). MicroRNA-27a induces mesangial cell injury by targeting of PPARγ, and its in vivo knockdown prevents progression of diabetic nephropathy. *Scientific reports*, *6*, 26072.

Wu, S. C., & Zhang, Y. (2010). Active DNA demethylation: many roads lead to Rome. *Nature reviews Molecular cell biology*, *11*(9), 607-620.

Xu, E., Kumar, M., Zhang, Y., Ju, W., Obata, T., Zhang, N., ... & Wheeler, M. B. (2006). Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system. *Cell metabolism*, *3*(1), 47-58.

Xu, Z., Jia, Q., Li, H., Wang, J., Zhao, Y., Chen, S., & Long, H. (2013). Effects of parthenolide on high glucose-induced cell proliferation, NF-κB activation and MCP-1 expression in rat mesangial cells. *Nan fang yi ke da xue xue bao= Journal of Southern Medical University*, *33*(10), 1471-1473.

Yan, Y. M., Fang, P., Yang, M. T., Li, N., Lu, Q., & Cheng, Y. X. (2015). Anti-DNcompounds from Cinnamomum cassia. *Journal of ethnopharmacology*, *165*, 141-147.

Yang, B. T., Dayeh, T. A., Kirkpatrick, C. L., Taneera, J., Kumar, R., Groop, L., ... & Ling, C. (2011). Insulin promoter DNAMecorrelates negatively with insulin gene expression and positively with HbA 1c levels in human pancreatic islets. *Diabetologia*, *54*(2), 360-367.

Yang, B. T., Dayeh, T. A., Volkov, P. A., Kirkpatrick, C. L., Malmgren, S., Jing, X., ... & Ling, C. (2012). Increased DNAMeand decreased expression of PDX-1 in pancreatic islets from patients with type 2 diabetes. *Molecular endocrinology*, *26*(7), 1203-1212.

Yang, J., Zhang, R., Zhao, H., Qi, H., Li, J., Li, J. F., ... & Zhang, T. (2022, August). Bioinspired copper single-atom nanozyme as a superoxide dismutase-like antioxidant for sepsis treatment. In *Exploration* (Vol. 2, No. 4, p. 20210267).

Yang, P., Feng, J., Peng, Q., Liu, X., & Fan, Z. (2019). AGEs: potential mechanism and therapeutic target in cardiovascular complications under diabetes. *Oxidative Medicine and Cellular Longevity*, *2019*.

Yang, S., Ma, C., Wu, H., Yuan, F., Yang, G., Yang, Q., ... & Kang, L. (2020). Tectorigenin attenuates DNby improving vascular endothelium dysfunction through activating AdipoR1/2 pathway. *Pharmacological research*, *153*, 104678.

Yang, X. J., & Seto, E. H. A. T. (2007). HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene*, *26*(37), 5310.

Yokoyama, S., Hiramatsu, J. I., & Hayakawa, K. (2002). Production of γ-aminobutyric acid from alcohol distillery lees by Lactobacillus brevis IFO-12005. *Journal of bioscience and bioengineering*, *93*(1), 95-97.

Yosri, H., El-Kashef, D. H., El-Sherbiny, M., Said, E., & Salem, H. A. (2022). Calycosin modulates NLRP3 and TXNIP-mediated pyroptotic signaling and attenuates DN progression in diabetic rats; An insight. *Biomedicine & Pharmacotherapy*, *155*, 113758.

Yu, S. H., Dubey, N. K., Li, W. S., Liu, M. C., Chiang, H. S., Leu, S. J., ... & Deng, W. P. (2016). Cordyceps militaris treatment preserves renal function in type 2 DN mice. *PLoS One*, *11*(11), e0166342.

Yuan, H., Reddy, M. A., Sun, G., Lanting, L., Wang, M., Kato, M., & Natarajan, R. (2012). Involvement of p300/CBP and epigenetic HAc in TGF-β1-mediated gene transcription in mesangial cells. *American Journal of Physiology-Renal Physiology*, *304*(5), F601-F613.

Zelmanovitz, T., Gross, J. L., Oliveira, J., & De Azevedo, M. J. (1998). Proteinuria is still useful for the screening and diagnosis of overt diabetic nephropathy. *Diabetes care*, *21*(7), 1076-1079.

Zhang, D., Ye, S., & Pan, T. (2019). The role of serum and urinary biomarkers in the diagnosis of early DN in patients with type 2 diabetes. *PeerJ*, *7*, e7079.

Zhang, H., Dai, X., Qi, Y., He, Y., Du, W., & Pang, J. J. (2015). HDACinhibitors in the treatment of retinal degenerative diseases: overview and perspectives. *Journal of ophthalmology*, *2015*.

Zhang, W., Gao, J., Lu, L., Bold, T., Li, X., Wang, S., ... & Tang, J. (2021). Intracellular GSH/GST antioxidants system change as an earlier biomarker for toxicity evaluation of iron oxide nanoparticles. *NanoImpact*, *23*, 100338.

Zhang, X., Guo, K., Xia, F., Zhao, X., Huang, Z., & Niu, J. (2018). FGF23 C-tail improves DNby attenuating renal fibrosis and inflammation. *BMC biotechnology*, *18*, 1-9.

Zhao, B., Li, H., Liu, J., Han, P., Zhang, C., Bai, H., ... & Jin, X. (2016). MicroRNA-23b targets Ras GTPase-activating protein SH3 domain-binding protein 2 to alleviate fibrosis and albuminuria in diabetic nephropathy. *Journal of the American Society of Nephrology*, *27*(9), 2597-2608.

Zhao, Q., Wan, Y., Wang, C., Wei, Q., Chen, H., Meng, X., & Yao, J. (2012). Regulatory mechanism of p38MAPK signaling pathway on renal tissue inflammation in chronic kidney disease and interventional effect of traditional Chinese medicine. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica*, *37*(12), 1700-1704

Zheng, Y., Tang, L., Huang, W., Yan, R., Ren, F., Luo, L., Zhang, L., 2015. Anti-inflammatory effects of ang-(1-7) in ameliorating HFD-induced renal injury through LDLr-SREBP2-SCAP pathway. PLoS One 10, 0136187

Zhong, Q., & Kowluru, R. A. (2011). Epigenetic changes in mitochondrial SODin the retina and the development of diabetic retinopathy. *Diabetes*, *60*(4), 1304-1313.

Zhong, Q., & Kowluru, R. A. (2013) (a). Epigenetic modification of Sod2 in the development of diabetic retinopathy and in the METABOLIC MEMORY: role of histone methylation. *Investigative ophthalmology & visual science*, *54*(1), 244-250.

Zhong, Q., & Kowluru, R. A. (2013) (b). Regulation of matrix metalloproteinase-9 by epigenetic modifications and the development of diabetic retinopathy. *Diabetes*, *62*(7), 2559-2568.

Zhong, X., Chung, A. C. K., Chen, H. Y., Dong, Y., Meng, X. M., Li, R., ... & Lan, H. Y. (2013). miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. *Diabetologia*, *56*(3), 663-674.

Zhou, T., Xu, H., Cheng, X., He, Y., Ren, Q., Li, D., ... & Huang, W. (2022). Sodium butyrate attenuates DKDpartially via histone butyrylation modification. *Mediators of inflammation*, *2022*.

Zhou, V. W., Goren, A., & Bernstein, B. E. (2011). Charting histone modifications and the functional organization of mammalian genomes. *Nature Reviews Genetics*, *12*(1), 7.

Zhou, W., Liu, Y., Hu, Q., Zhou, J., & Lin, H. (2022). The landscape of immune cell infiltration in the glomerulus of diabetic nephropathy: evidence based on bioinformatics. *BMC nephrology*, *23*(1), 1-11.

Zhou, X., He, L., Zuo, S., Zhang, Y., Wan, D., Long, C., ... & Yin, Y. (2018). Serine prevented HFD-induced oxidative stress by activating AMPK and epigenetically modulating the expression of glutathione synthesis-related genes. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1864*(2), 488-498.

Zhu, D., Wang, L., Zhou, Q., Yan, S., Li, Z., Sheng, J., & Zhang, W. (2014). (+)-Catechin ameliorates DNby trapping methylglyoxal in type 2 diabetic mice. *Molecular nutrition & food research*, *58*(12), 2249-2260.

Zhu, J., Li, D., Yu, T., & Zhu, D. (2022). Optical angiography for diabetes-induced pathological changes in microvascular structure and function: An overview. *Journal of Innovative Optical Health Sciences*, *15*(01), 2230002.

Ziyadeh, F. N., & Sharma, K. (2003). Overview: combating diabetic nephropathy. *Journal of the American Society of Nephrology*, *14*(5), 1355-1357.

THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22-Issue-37-December -2019

Esculetin and its similar compounds: Implications in binding Sirt1 and treating diabetic nephropathy

Kriti Kushwaha and Jeena Gupta
Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University (LPU), Jalandhar-Delhi G.T. Road, Phagwara, Punjab (India) - 144411
Phone: +91-1824-404404, Fax: +91-1824-506111,
E-mail: jeena.20104@lpu.co.in

Abstract:

Diabetic nephropathy(DN)occurs as a result of prolonged high levels of glucose in diabetic patients. It is diagnosed primarily upon the presence of microalbuminuria (MA). Factors like dyslipidemia, high blood sugar levels, free radicals, smoking, advanced glycation end products, environmental, and genetic factors play an important role in the progression of diabetic nephropathy. Many reports have highlighted the role of genetics and epigenetics including histone modifications in the progression of DN. Histone acetyl transferases (HATs) and Histone deacetylases (HDACs)which regulates histone acetylation levels are known to play an important role in chromatin remodelling, affecting various gene transcription that is related to metabolism, apoptosis, immunity, and angiogenesis. HDAC inhibitors are now emerging as new therapeutic target. Treatment methods still are not so prevalent in ameliorating DN; some of them include controlling hypertension, controlling high blood sugar level, cessation of smoking and drinking, and renal replacement. The number of side effects associated with synthetic drugs makes researchers look for alternatives that are less toxic. Phytochemicals can be a good alternative to these synthetic drugs as they are relatively safe and have multiple interactive targets. In order to fish out potent phytochemicals,a thorough review was done on current existing literature, and Esculetin was chosen. Structurally similar compounds were found by doing structural similarity searches with filter 80%. Top 3 similar compounds were chosen and further docked with human SIRT1 protein. Docking was done to identify potent compound for treating DN and similar binding energies proved similar compounds may have the potential to ameliorate DN.

Keywords: *Nephropathy, Microalbuminuria, Phytochemicals, Epigenetics, Histone modifications, HDAC Inhibitors, Docking.*

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

Metabolic memory and diabetic nephropathy: Beneficial effects of natural epigenetic modifiers

Kriti Kushwaha [a], Sandeep Sharma [b], Jeena Gupta [a, *]

[a] Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, 144411, India
[b] Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Lovely Professional University, Phagwara, Punjab, 144411, India

ARTICLE INFO

Article history:
Received 7 November 2019
Accepted 13 January 2020
Available online 16 January 2020

Keywords:
Diabetes
Diabetic nephropathy
Metabolic memory
Epigenetics
Phytochemical
Oxidative stress

ABSTRACT

Nephropathy is one of the most frequent complications of chronic diabetes. The main reason for nephropathy despite being hyperglycemia, but it progresses even after good glycemic control has been achieved in diabetic patients. The effects of prior exposure to high blood glucose conditions depend upon the severity and duration of this exposure, indicating a "metabolic memory" phenomenon. Hyperglycemia not only increases oxidative stress but is also alleged to start several biochemical anomalies and alter gene expression associated with metabolic homeostasis. High glucose levels induce epigenetic modifications that alter gene expression without changing DNA sequences. These epigenetic modifications have shown to be reversible and have the potential to cease adverse effects if good glycemic control is achieved from initiation of diabetes. However, if good glycemic control is not achieved for months, these modifications stand firm to reversals. Therapies and drugs have been in use to prevent epigenetic modifications and oxidative stress, which also helped in ameliorating diabetic nephropathy. But these synthetic drugs are loaded with side effects like increased body weight, kidney dysfunction etc. So phytochemicals are emerging as alternatives and many of them have already been used to treat nephropathy. But still, there is rigorous need to evaluate phytochemicals which can regulate epigenetic events and have the potential to decelerate the further progression of these life-threatening diseases. In this review article we discuss the potential epigenetic modifiers from plants that can erase metabolic memory and can thus be protective against diabetic nephropathy.

Diabetic Nephropathy: Pathogenesis to Cure

Authors: Kushwaha, Kriti; Kabra, Uma; Dubey, Rupal; Gupta, Jeena
Source: Current Drug Targets, Volume 23, Number 15, 2022, pp. 1418-1429(12)
Publisher: Bentham Science Publishers
DOI: https://doi.org/10.2174/1389450123666220820110801

< previous article | view table of contents | next article > ADD TO FAVOURITES

Abstract | References | Citations | Supplementary Data

Diabetic nephropathy (DN) is a leading cause of end-stage renal disorder (ESRD). It is defined as the increase in urinary albumin excretion (UAE) when no other renal disease is present. DN is categorized into microalbuminuria and macroalbuminuria. Factors like high blood pressure, high blood sugar levels, genetics, oxidative stress, hemodynamic and metabolic changes affect DN. Hyperglycemia causes renal damage through activating protein kinase C (PKC), producing advanced end glycation products (AGEs) and reactive oxygen species (ROS). Growth factors, chemokines, cell adhesion molecules, inflammatory cytokines are found to be elevated in the renal tissues of the diabetic patient. Many different and new diagnostic methods and treatment options are available due to the increase in research efforts and progression in medical science. However, until now, no permanent cure is available. This article aims to explore the mechanism, diagnosis, and therapeutic strategies in current use for increasing the understanding of DN.

Keywords: Diabetic nephropathy; macroalbuminuria; microalbuminuria; pathogenesis; renal damage; urinary albumin excretion
Document Type: Review Article
Publication date: November 1, 2022

› More about this publication?

Biochimie
Volume 202, November 2022, Pages 146-158

Targeting epigenetic regulators for treating diabetic nephropathy

Kriti Kushwaha [a], Sourbh Suren Garg [b], Jeena Gupta [b]

https://doi.org/10.1016/j.biochi.2022.08.001

Abstract

Diabetes is accompanied by the worsening of kidney functions. The reasons for kidney dysfunction mainly include high blood pressure (BP), high blood sugar levels, and genetic makeup. Vascular complications are the leading cause of the end-stage renal disorder (ESRD) and death of diabetic patients. Epigenetics has emerged as a new area to explain the inheritance of non-mendelian conditions like diabetic kidney diseases. Aberrant post-translational histone modifications (PTHMs), DNA methylation (DNAme), and miRNA constitute major epigenetic mechanisms that progress diabetic nephropathy (DN). Increased blood sugar levels alter PTHMs, DNAme, and miRNA in kidney cells results in aberrant gene expression that causes fibrosis, accumulation of extracellular matrix (ECM), increase in reactive oxygen species (ROS), and renal injuries. Histone acetylation (HAc) and histone deacetylation (HDAC) are the most studied epigenetic modifications with implications in the occurrence of kidney disorders. miRNAs induced by hyperglycemia in renal cells are also responsible for ECM accumulation and dysfunction of the glomerulus. In this review, we highlight the role of epigenetic modifications in DN progression and current strategies employed to ameliorate DN.

Diabetes Research and Clinical Practice
Volume 200, June 2023, 110691

Association between obesity, inflammation and insulin resistance: Insights into signaling pathways and therapeutic interventions

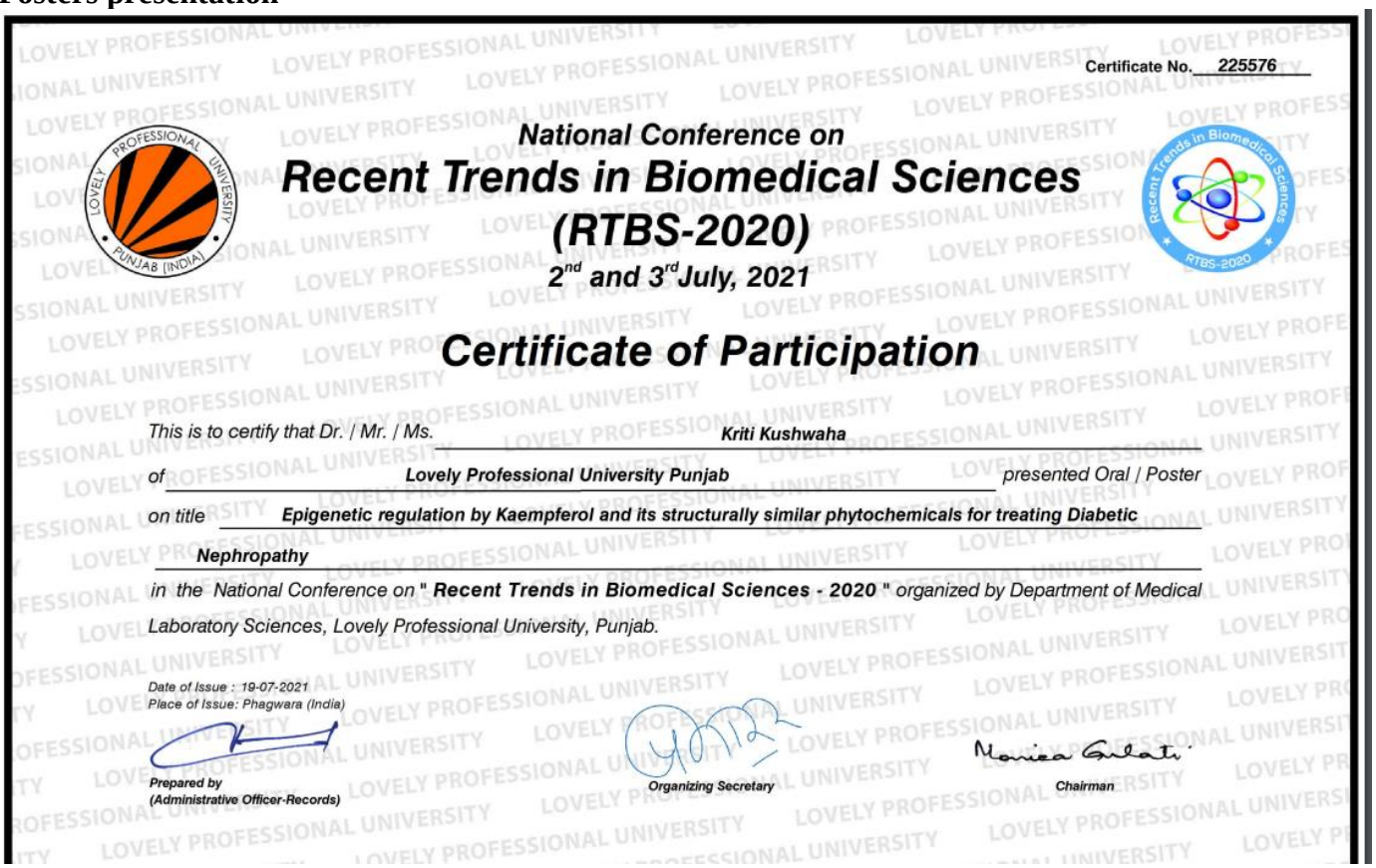
Sourbh Suren Garg [a], Kriti Kushwaha [b 1], Rupal Dubey [c 1], Jeena Gupta [a]

https://doi.org/10.1016/j.diabres.2023.110691

Abstract

Obesity, a metabolic disorder, is becoming a worldwide epidemic that predominantly increases the risk for various diseases including metabolic inflammation, insulin resistance, and cardiovascular diseases. However, the mechanisms that link obesity with other metabolic diseases are not completely understood. In obesity, various inflammatory pathways that cause inflammation in adipose tissue of an obese individual become activated and exacerbate the disease. Obesity-induced low-grade metabolic inflammation perturbates the insulin signaling pathway and leads to insulin resistance. Researchers have identified several pathways that link the impairment of insulin resistance through obesity-induced inflammation like activation of Nuclear factor kappa B (NF-κB), suppressor of cytokine signaling (SOCS) proteins, cJun-N-terminal Kinase (JNK), Wingless-related integration site (Wnt), and Toll-like receptor (TLR) signaling pathways. In this review article, the published studies have been reviewed to identify the potential and influential role of different signaling pathways in the pathogenesis of obesity-induced metabolic inflammation and insulin resistance along with the discussion on potential therapeutic strategies. Therapies targeting these signaling pathways show improvements in metabolic diseases associated with obesity, but require further testing and confirmation through clinical trials.

Posters presentation



Certificate No. 225576

National Conference on

Recent Trends in Biomedical Sciences (RTBS-2020)

2nd and 3rd July, 2021

Certificate of Participation

This is to certify that Dr. / Mr. / Ms. **Kriti Kushwaha**

of **Lovely Professional University Punjab** presented Oral / Poster

on title **Epigenetic regulation by Kaempferol and its structurally similar phytochemicals for treating Diabetic Nephropathy**

in the National Conference on " **Recent Trends in Biomedical Sciences - 2020** " organized by Department of Medical Laboratory Sciences, Lovely Professional University, Punjab.

Date of Issue : 19-07-2021
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

Organizing Secretary

Chairman

3ICP2022ORAL PRESENTATION 23
Serial No. _____

सत्यमेव जयते
IPC

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. **Kriti Kushwaha** has successfully participated as Delegate & Presented Poster/ Oral Presentation on **To Evaluate epigenetic modifications of short- chain fatty acids for therapeutical application against Type I diabetes** in the 3rd International Conference of Pharmacy (ICP-2022) on the Theme of "Practice, Promotion & Publication of Innovation : A Way of Transforming Health" held on 09th & 10th November 2022 organized by School of Pharmaceutical Sciences in a collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna
National Hon. Gen. Secretary, IPA

Dr. Bimlesh Kumar
Organizing Secretary

Dr. T.V. Naryana
National President IPA

Dr. Monica Gulati
LOC Chairperson

Sri Herbasia Biotech

Tishk International University, Iraq

Certificates No. 173603

Certificate of Merit

This is to certify that Prof./Dr./Mr./Ms. KRITI KUSHWAHA

has **Presented E poster on** Epigenetic modifying potential of lipoic acid: Implications in curing diabetes. **and awarded for best Poster presentation in International Conference of Pharmacy (ICP-2019) on the theme of " Pharmacy: Realigning the focus on health"** held on 13 -14th September 2019 organized by School of Pharmaceutical Sciences, Lovely Professional University, Punjab in a collabration with Indian Pharmacy Graduates' Assocaition (IPGA) Phagwara chapter.

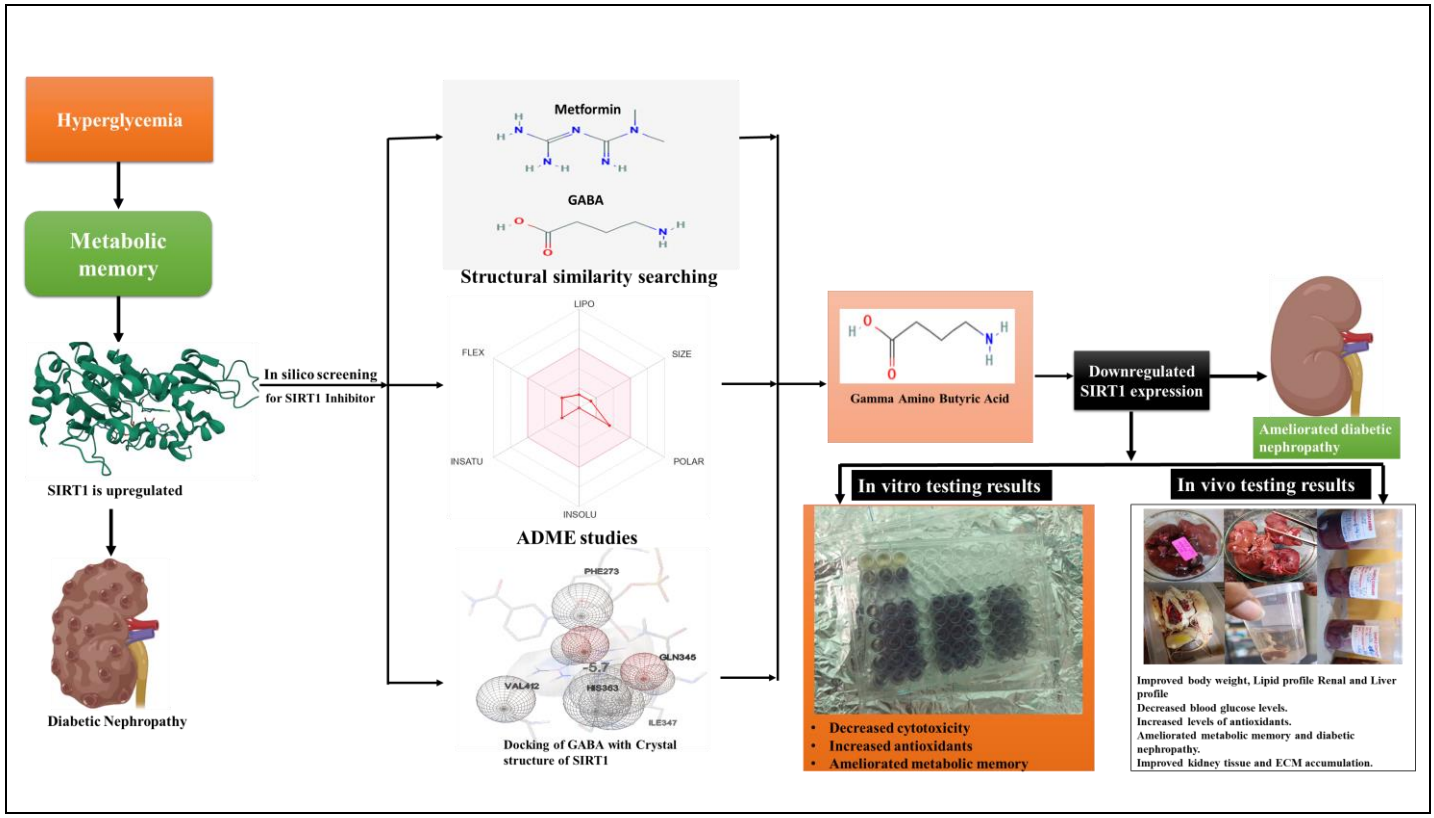
Date of Issue: 14-09-2019
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

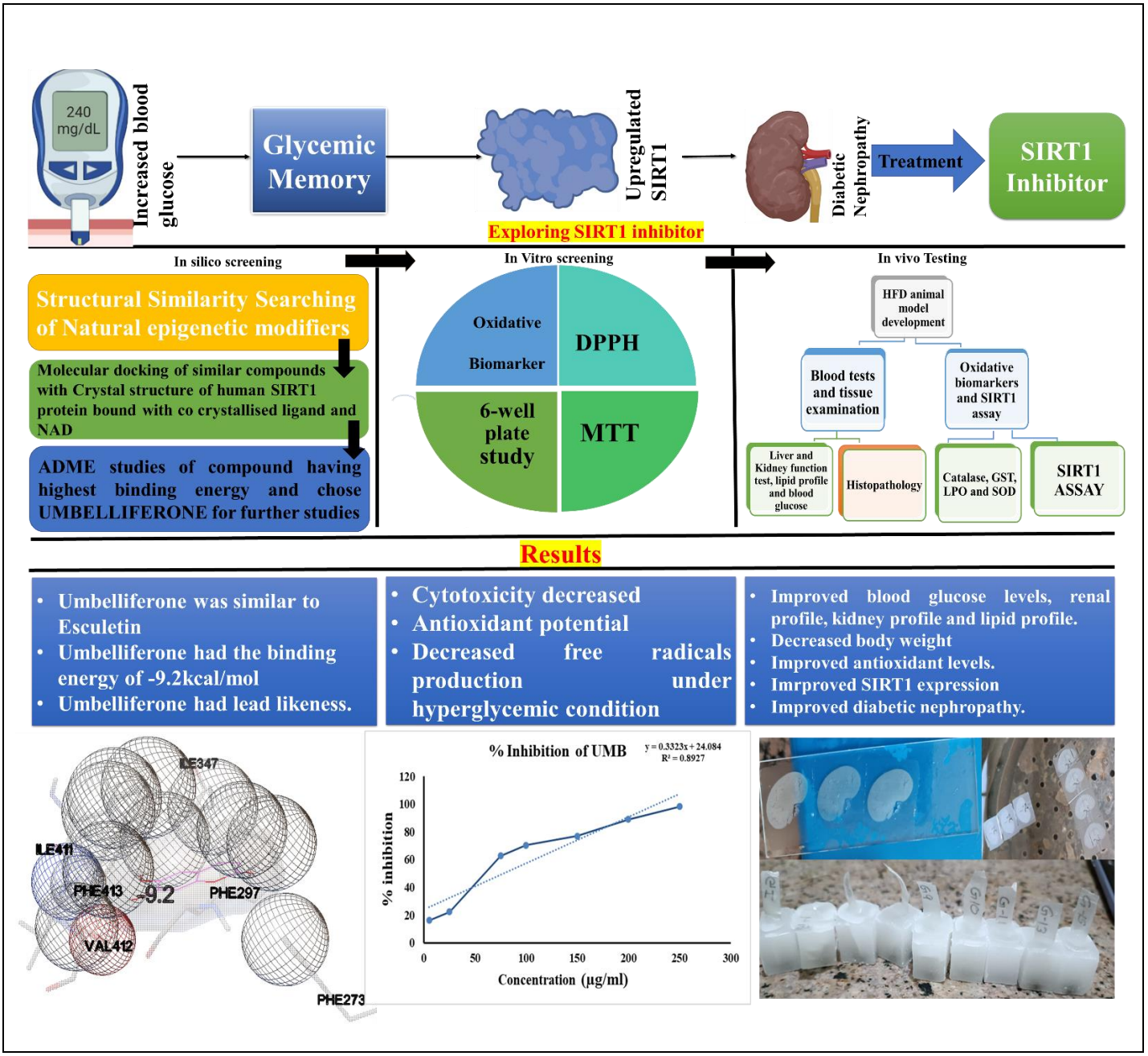
Dr. Bimlesh Kumar
Organizing Secretary

Dr. Monica Gulati
Chairperson, Local Organizing Committee

Graphical abstract



Caption: Role of GABA in protecting against hyperglycemia-induced Metabolic Memory and Diabetic Nephropathy



Caption: Role of UMBelliferone in protecting against hyperglycemia-induced Metabolic Memory and Diabetic Nephropathy

Thesis

ORIGINALITY REPORT

10%	5%	8%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.nature.com Internet Source	<1%
2	docplayer.net Internet Source	<1%
3	www.researchgate.net Internet Source	<1%
4	link.springer.com Internet Source	<1%
5	Systems Biology of Free Radicals and Antioxidants, 2014. Publication	<1%
6	Shamini Sharma, Jeena Gupta, Pranav Kumar Prabhakar, Pawan Gupta, Preeti Solanki, Anuradha Rajput. " Phytochemical Repurposing of Natural Molecule: Sabinene for Identification of Novel Therapeutic	<1%