DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

The role of certain traditional plants (*Trigonella foenum-graecum* L. and *Equisetum arvense* L.), in the prophylaxis and management of obesity, type 2 diabetes mellitus, and diabetic cardiomyopathy

by Andrea Badale

Supervisor: Dr. Rita Kiss

UNIVERSITY OF DEBRECEN
KÁLMÁN LAKI DOCTORAL SCHOOL

DEBRECEN, 2024

TABLE OF CONTENTS

Abbreviations

ACAA2	Acetyl-coA Acyltransferase 2
ACEi	Angiotensin converting enzyme inhibitors
ADCY3	Adenylate cyclase 3
AGE	Advanced glycation end product
AGIs	Alpha-glucosidase inhibitors
AKR1B1	Aldo-keto reductase family 1 member b
AMPK	Activated protein kinase
ANOVA	Analysis of variance
ARB	Angiotensin receptor blockers
ASC	Adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain
ATF6	Activating transcription factor 6
BCA	Bicinchoninic acid
BG	Blood glucose
BMI	Body mass index
BP	Blood pressure
BSA	Bovine serum albumin
CAD	Coronary artery disease
CHO	Consistent carbohydrate
CircRNA	Circular ribonucleic acid
COPD	Chronic obstructive pulmonary disease
CP	Crude protein
CR	Calorie restriction
CRP	C-reactive protein
CT	Computerized tomography
CV	Cardiovascular
CVD	Cardiovascular disease
DC	Diabetic control
DCM	Diabetic cardiomyopathy
DG	Diosgenin
DIO	Diet induced obese
DM	Diabetes mellitus

DNA	Deoxyribonucleic acid
DPP-4	Dipeptidyl-peptidase-4
EC	Expiratory capacity
ECM	Extracellular matrix
ECs	Endothelial cells
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
eIF2α	Eukaryotic initiation factor 2
eNOS	Endothelial nitric oxide synthase
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
ERK1	Extracellular signal-regulated kinase 1
ET-1	Endothelin 1
EU	European union
Eur.Ph	European pharmacopoeia
FDA	Food and Drug Administration
FFA	Fundus fluorescein angiography
FOXO	Forkhead box transcription factors
FOXO3a	Forkhead Transcription Factor O Subfamily Member 3a
F.R.X	Romanian Pharmacopoeia edition X
GH	Growth hormone
GLUT-4	Glucose transport-4
GLP-1	Glucagon-like peptide-1
GLP-1-Ras	Glucagon-like peptide-1 receptor agonists
HbA1c	Glycated haemoglobin
HC	Healthy control
HDACs	Histone deacetylases
HF	Heart failure
HFD	High-fat diet
HPLC	High performance liquid chromatography
50HT	Treatment with 50 mg/kg horsetail extract
100HT	Treatment with 100 mg/kg horsetail extract
200HT	Treatment with 200 mg/kg horsetail extract

IDF	International diabetes federation
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor-1
IL-6	Interleukin-6
INR	International normalized ratio
IR	Insulin resistance
IRE1α	Inositol-requiring enzyme 1
IRS-1	Insulin receptor substrate 1
ITT	Insulin tolerance test
IU	International unit
JNK	Jun N-terminal kinase
KO	Knock Out
LD	Lactate dehydrogenase
LEP	Leptin
LEPR	Leptin receptor
LV	Left ventricle
MAPK	Mitogen-activated protein kinases
MC4R	Melanocortin 4 receptor
MCH	Melanin concentrating hormone
MCHR1	Melanin concentrating hormone receptor 1
MetS	Metabolic syndrome
MiRNA	Micro-ribonucleic acid
MMP-9	Matrix metalloproteinase-9
MRI	Magnetic resonance imaging
MyoD	Myoblast determination protein 1
NaF	Sodium fluoride
NAD+	Nicotinamide adenine dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NCDs	Non-communicable diseases
NOX	NADPH oxidase
Nox2	NADPH oxidase 2
NPH	Normal pressure hydrocephalus
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells

NRF	Nuclear respiratory factor
Nrf2	Nuclear factor erythroid 2–related factor 2
NLRP3 inflammasome	NOD-like receptor protein 3 inflammasome
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NF-jB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OGTT	Oral glucose tolerance test
O-GlcNAc	O-linked β-N-acetyl glucosamine
OSA	Obstructive sleep apnoea
p38MAPK	p38 mitogen-activated protein kinases
PAD	Peripheral artery disease
PCOS	Polycystic ovarian syndrome
PCSK1	Proprotein convertase 1
PDE5/PDE5i	Phosphodiesterase-5/Phosphodiesterase 5 Inhibitors
PER2	Period Circadian Regulator 2
PERK	Protein kinase R
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator
Ph.Hg	Hungarian pharmacopoeia
PKC	Protein kinase C
PKCβ	B isoform of protein kinase C
PMSF	Phenylmethylsulfonyl fluoride
POMC	Pro-opiomelanocortin
PPARs	Peroxisome proliferator-activated receptor
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PTP1B	Protein tyrosine phosphatase 1B
RAA	Rapid-acting insulin analogues
RAAS	Renin-angiotensin-aldosterone system
RIA	Radioimmunoassay
RM AFE	Rodent maintenance at water fuel energy
ROS	Reactive oxygen species
SCT	Stem cell transplantation
SEM	Scanning electron microscope
SERCA2a	Sarcoplasmic/endoplasmic reticulum calcium ATPase
SGLT	Sodium–glucose cotransporter

SIE	Italian Society of Endocrinology
SIO	Italian Society of Obesity
SGLT2	Sodium–glucose cotransporter-2
SGLT2i	Sodium-glucose cotransporter-2 inhibitors
Sir2	Silent information regulation-2
SIRT1	Sirtuin 1
SIRT3	Sirtuin 3
SIRT5	Sirtuin 5
SIRT6	Sirtuin 6
SNPs	Single-nucleotide polymorphism
SNS	Sympathetic nervous system
SOD1, SOD2	Oxidative stress factors
STZ	Streptozotocin
SUs	Sulfonylurea
SUR1	Sulfonylurea receptor 1
T3	Triiodothyronine
T4	Thyroxine
TBS-T	Tris-buffered saline with Tween 20
TCH	Tetrahydrocannabinol
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TFG	*Trigonella foenum-graecum* L.
TGF-β	Transforming growth factor-β
TNF-α	Tumour necrosis factor α
TZDs	Thiazolidinediones
TRPC3	Transient receptor potential channel 3
UHPLC	Ultra-high performance liquid chromatography
UPLC-DAD	Ultra-performance liquid chromatography
UK	United kingdom
UPR	Unfolded protein response
US	United states
URAA	Ultra rapid-acting insulin analogues
VLCKD	Very low-calorie ketogenic diets

WAT	White adipose tissue
WHO	World health organisation
Zn	Zinc

1 Introduction

Obesity is one of the outstanding health challenges of the 21st century, which is escalating as an epidemic across all age groups in both developed and developing nations Recognized by the World Health Organization (WHO) as a worldwide epidemic and the foremost health issue, obesity carries a heightened risk of developing various co-morbidities including type 2 diabetes (T2DM) mellitus, cardiovascular diseases (CVD), cerebrovascular complications, sleep disorders, orthopaedic disorders, and cancer. Despite its alarming prevalence, obesity remains a preventable cause of mortality, attributed primarily to lifestyle and dietary modifications rather than genetic predispositions.

Diabetes represents the most prevalent metabolic disorder worldwide, with its incidence and prevalence on a continual rise. T2DM represents the predominant form, distinguished by hyperglycaemia, IR, and relative insulin deficiency. Both genetic predisposition and environmental factors can play role in T2DM development, but environmental influences, lifestyle choices, and related comorbidities hold greater significance. T2DM is characterized by inadequate insulin secretion response from pancreatic β-cells to hyperglycaemia, coupled with diminished insulin sensitivity in insulin-sensitive tissues such as adipose tissue, muscle, and hepatic tissues, leading to IR. Diabetic cardiomyopathy emerges as a consequential complication of diabetes, impacting cardiac muscle structure and function. Its aetiology involves hyperglycaemia-induced oxidative stress, fibrosis, and apoptosis within the myocardium.

Despite extensive research efforts aimed at preventing and treating obesity and T2DM, effective treatments remain elusive. Consequently, ongoing investigations focus on uncovering novel signalling pathways implicated in the pathophysiology of diabetes mellitus. Such endeavours hold promise for the development of advanced and efficacious antidiabetic therapeutics, or interventions capable of retarding the progression of diabetes or mitigating its complications.

Sirtuins, a family of NAD+-dependent protein deacetylases, exert influence over a multitude of metabolic pathways. and diabetes mellitus. In tissues and organs implicated in lipid metabolism, such as the liver, both white and brown adipose tissue, and skeletal muscle, sirtuins regulate the synthesis, storage and use of lipids directly and indirectly by regulating insulin secretion. Elevated expression of SIRT1 specifically in pancreatic β-cells enhances insulin secretion and shields the β-cells from damage-induced apoptosis. As potential therapeutic targets, they hold promise in addressing obesity and diabetes mellitus. Activation of SIRT1, a key player in established signalling pathways of diabetic cardiomyopathy, confers protective effects against

oxidative stress, inflammatory cascades, and apoptosis, which underlie pathomechanisms in conditions such as obesity, diabetes mellitus, and CVD.

In the field of medical sciences, there is an interest in studying the therapeutic effects of different plant species, as supportive treatment of obesity and T2DM.

The literature reports the presence of more than 800 plant species with a potential hypoglycaemic effect, which may mean the expansion of alternative options for the treatment of T2DM [1]. Among these, Fenugreek (*Trigonella foenum-graecum* L. - TFG) as a prominently cited plant with therapeutic efficacy in lipid and glucose metabolism. It exhibits insulin-sensitizing properties, antioxidant effects, and contributes to maintaining energy balance [2]. Horsetail (*Equisetum arvense* L.) is a medicinal plant with a rich history of use dating back to ancient times, a tradition that persists unbroken to the present day. Horsetail extracts boast rich reservoirs of phenolic compounds, flavonoids, and phenolic acids. Numerous studies have outlined various biological effects of field horsetail extracts, such as antibacterial and antifungal activities, antioxidant properties, anti-inflammatory effects, neuroprotective and cardioprotective benefits, as well as antiproliferative characteristics.

1.1 Obesity

1.1.1 Incidence and prevalence of obesity

Obesity stands as one of the most pervasive global health challenges of the 21st century, posing heightened risks of mortality and morbidity. Defined by excessive fat accumulation detrimental to health, obesity in adults is typically characterized using parameters such as BMI (Body Mass Index), waist circumference, and waist-to-hip ratio. A BMI exceeding 30 signifies obesity [3] (Table I.).

Table I. *BMI classification [3, 4]*

Body mass Index (BMI) kg/m^2	Classification	Risk of co-morbidities
<18.5	Underweight	Low
18.5-.24.9	Normal Weight	Average
25.0-29.9	Overweight	Mildly increased
30.0-34.9	Obesity Class I	Moderate
35.0-39.9	Obesity Class II	Severe
≥40	Obesity Class III (Extreme obesity)	Very severe

In 2016, the WHO approximated the global count of overweight adults at around 1.9 billion, with 650 million classified as obese (BMI ≥30 kg/m^2). This statistic encompassed 340 million adolescents and 39 million children, signifying that 39% of adults were overweight, and 13% were obese. In 2022, one in every eight individuals worldwide was grappling with obesity. Over the years, there has been a stark rise in global adult obesity, more than doubling since 1990, while adolescent obesity has increased fourfold. By 2022, a staggering 2.5 billion adults were classified as overweight, with 890 million of them coping with obesity. Accordingly, among adults 43% were overweight, with 16% living with obesity. Additionally, in 2022, approximately 37 million children and toddlers younger than 5 years were identified as overweight. Moreover, in age range of 5–19 years 390 million children and adolescents were considered overweight, with 160 million among them classified as living with obesity.

Regrettably, epidemiological projections from 2019 indicate that by 2030, approximately 57.8% of the global population will be classified as overweight or obese [5].

1.1.2 Ethiology of obesity

Certain researchers underscore that variations in obesity prevalence among different population groups stem from environmental factors, particularly dietary habits and decreased physical activity levels. Both endogenous (genetic) and exogenous factors (such as diet and physical activity) can impact the development and management of obesity. Additionally, some epidemiological studies view obesity as a chronobiological condition influenced by chronic sleep disturbances. Sleep deprivation affects peripheral hunger regulators, leading to elevated blood levels of ghrelin and diminished levels of the satiety hormone leptin [6].

Recent studies suggest that high-fat intake, particularly saturated fats, may disrupt circadian rhythms in response to light, leading to metabolic changes resembling those observed in human metabolic syndrome (MetS), including obesity and IR [6]. Additionally, certain psychological issues such as melancholic depression, which can perturb circadian rhythms, have been associated with obesity [7].

Genetic variations in human clock genes, such as SNPs rs3749474, rs4580704, and rs1801260 (3111T4C), have been linked to BMI and energy intake, while (Period Circadian Regulator 2) PER2 SNPs rs2304672C4G and rs4663302C4T are linked to abdominal obesity [8].

Obesity, a disorder influenced by genetics, can be categorized into monogenic and polygenic forms, involving gene-environment interactions [9]. Syndromes commonly associated with obesity include Albright's hereditary osteodystrophy, Bardet-Biedl syndrome, Fragile X syndrome, Prader-Willi syndrome, and Wilms-tumour-aniridia-syndrome [9].

Regarding non-syndromic monogenic obesity, numerous genes play a role in regulating energy homeostasis via the leptin-melanocortin pathway. Homozygous loss-of-function mutations in genes such as ADCY3, LEP, LEPR, MC4R, PCSK1, and POMC can lead to monogenic obesity, influenced in part by environmental factors. For instance:

- MC4R gene mutations may result in hyperphagia, elevated lean mass, and linear growth.
- PCSK1 mutations can disrupt POMC processing, leading to obesity accompanied by postprandial hypoglycaemia, glucocorticoid deficiency with hypogonadotropic hypogonadism [9].
- The melanin-concentrating hormone (MCH) and its receptor MCHR1 play roles in regulating energy balance. MCH overexpression in mice and MCHR1 inactivation can predispose individuals to obesity and IR [10].

Furthermore, gut hormones have emerged as crucial regulators of energy balance, influencing eating behaviour and weight loss post-Roux-en-Y gastric bypass and sleeve gastrectomy.

Future pharmacotherapies for obesity are expected to focus on gut hormones and their receptors [11].

1.1.3 Complications of obesity

The association between obesity and its complications is mediated by the insulin-insulin-like growth factor (IGF) axis and chronic low-grade inflammatory processes (Figure 1.).

Insulin/IGF Axis

Obesity is initially characterized by hyperinsulinemia followed by IR, which can lead to chronic complications including CVD and diabetes. The metabolism of insulin is intricately linked with the IGF system. Hyperinsulinemia can result in elevated levels of free active insulin-like growth factor-1 (IGF-1) and upregulation of hepatic IGF-1 synthesis.

The interaction between insulin and the IGF axis may potentially link obesity to a heightened risk of certain cancers. These cancers include oesophageal adenocarcinoma, endometrial cancer pancreatic cancer, postmenopausal breast cancer, and renal cell carcinoma. [12].

Biomarkers of Inflammation

Obesity is linked with chronic low-grade systemic inflammation, which is believed to contribute significantly to the development of IR. This inflammatory response in obesity is driven by the secretion of pro-inflammatory adipokines such as leptin and resistin, along with a decrease in the production of anti-inflammatory adiponectin. Leptin has been implicated in mediating the heightened risk of CVD associated with obesity, including hypertension. Resistin, on the other hand, contributes to obesity-related IR and is involved in various pathological processes resulting in CVD, including angiogenesis, endothelial dysfunction, smooth muscle cell dysfunction, thrombosis, and as well as promoting cancer risk.

In individuals with obesity, adipose tissue releases pro-inflammatory cytokines like tumour interleukin-6 (IL-6) and necrosis factor α (TNF-α), which stimulate the liver to secrete acute phase proteins like C-reactive protein (CRP) [13]. CRP is extensively studied as an inflammatory biomarker in disease risk assessment. Increased CRP levels have been associated with a higher risk of coronary heart disease, ischemic stroke, vascular and non-vascular mortality, and mortality from various cancers [12].

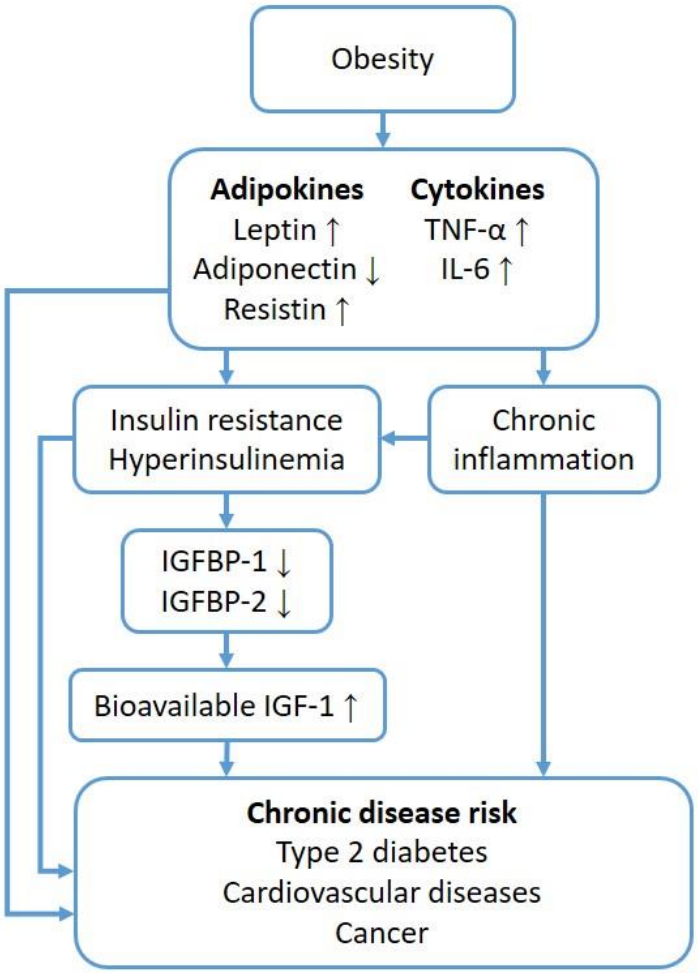

Figure 1. *Several pathways proposed to link obesity with risk of chronic diseases. [12]*

Another crucial factor underlying the complications of obesity is the gut microbiome. The gut microbiota modulates host metabolism, impacting energy regulation and metabolic inflammation associated with obesity, fat deposition, and IR [14]. Gut microbiota can serve as a bridge between genetic predisposition and environmental factors in the development of obesity [14].

The majority of individuals with T2DM are obese. The association between T2DM and obesity arises from the heightened production of adipocytokines in obesity, which triggers inflammation and insulin resistance. IR results in elevated blood glucose levels and heightened fat accumulation in adipose tissue [15].

The most prevalent cardiac complication in obese individuals is dilated cardiomyopathy, which can lead to fatal arrhythmias [15].

Hypertension stands out as the primary complication associated with obesity. BMI plays a significant role in influencing blood pressure, with studies demonstrating that weight loss can effectively lower blood pressure levels. Among the key factors of obesity-related hypertension

are sodium and water reabsorption disorders in the kidneys, pressure natriuresis and increased activation of the sympathetic nervous system [16, 17].

Obesity is linked to dyslipidaemia, characterized by elevated levels of low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglycerides (TG), and reduced levels of high-density lipoprotein cholesterol (HDL-C) [15].

Regarding the neurological complications, is it well known that increased BMI is linked to dysregulations of lipid metabolism, impaired glucose tolerance/diabetes, and hypertension, all of which are associated with a higher risk of dementia and Alzheimer's disease [15].

Obesity is recognized as a major risk factor for several types of malignant tumours, including breast, colorectal, endometrial, renal and oesophageal cancers. Additionally, it is associated with an increased risk of gallbladder, gastric, pancreatic cancer, and leukaemia [15].

Obstructive sleep apnoea (OSA) is prevalent in obese individuals and is associated with various health problems such as systemic inflammation, liver dysfunction, hypertension, IR, and dyslipidaemia. In children, OSA can result in failure to thrive, behavioural issues, diminished intellectual function, and heightened CV morbidity. Although obesity is linked to asthma, the exact mechanism remains unclear [15].

Obesity is associated with impaired immune function, increasing susceptibility to infections such as those post-surgery, urinary tract infections, nosocomial infections, and skin infections. Additionally, obese individuals may have reduced responses to vaccines.

Obesity is considered risk factor for various gastrointestinal disorders, including non-alcoholic fatty liver disease (NAFLD), gallbladder disease, pancreatitis, and gastroesophageal reflux disease (GERD). Weight loss interventions have been effective in preventing or treating these conditions [15].

Obesity is linked to a higher risk of urinary incontinence in women, kidney stones, and obesity-related nephropathy [15].

In men, obesity is linked to lower sperm count and higher rates of erectile dysfunction. Conversely, in women, obesity is known to decrease fertility, compromise outcomes following fertility treatments, and elevate the likelihood of pregnancy loss. Additionally, obesity is associated with a higher prevalence of Polycystic Ovarian Syndrome (PCOS) in women [15].

Reduced physical activity due to obesity increases the risk of osteoarthritis, inflammatory and mechanical joint conditions in the knees and hands. Obesity is also the most significant risk factor for gout [15].

Depression is particularly prevalent among women and young individuals affected by obesity [15]. The main complications of obesity are shown is Figure 2.

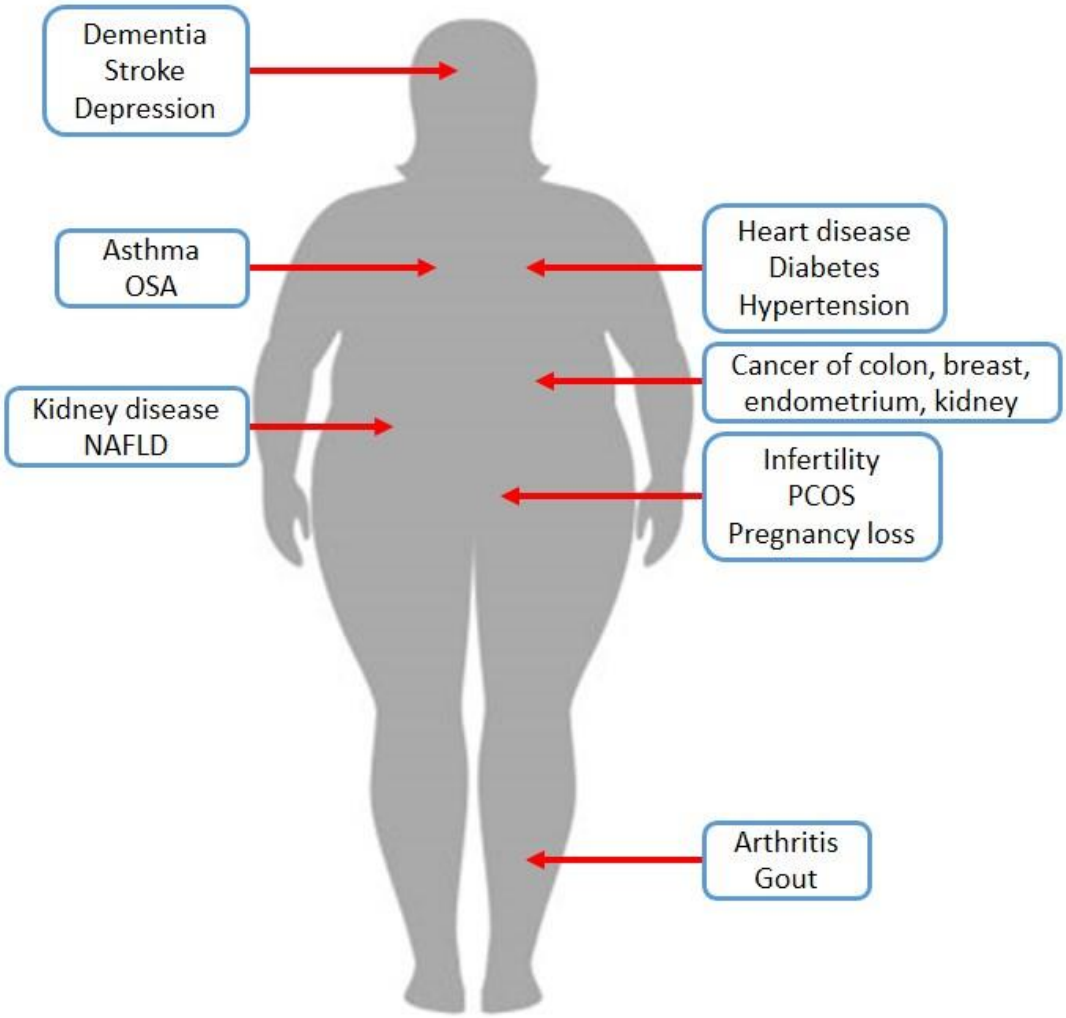

Figure 2. *Complications of obesity [3].*

1.1.4 **Therapeutic strategies in obesity**

Therapeutic interventions for obesity primarily include dietary adjustments, exercise regimens, and behavioural modifications. When these methods prove ineffective, pharmacotherapy is considered, particularly for individuals with a BMI ≥ 30 and at least one associated CV risk factor such as hypertension, hyperlipidaemia, or T2DM. If pharmacological interventions are unsuccessful, bariatric surgery becomes the ultimate option (Figure 3.).

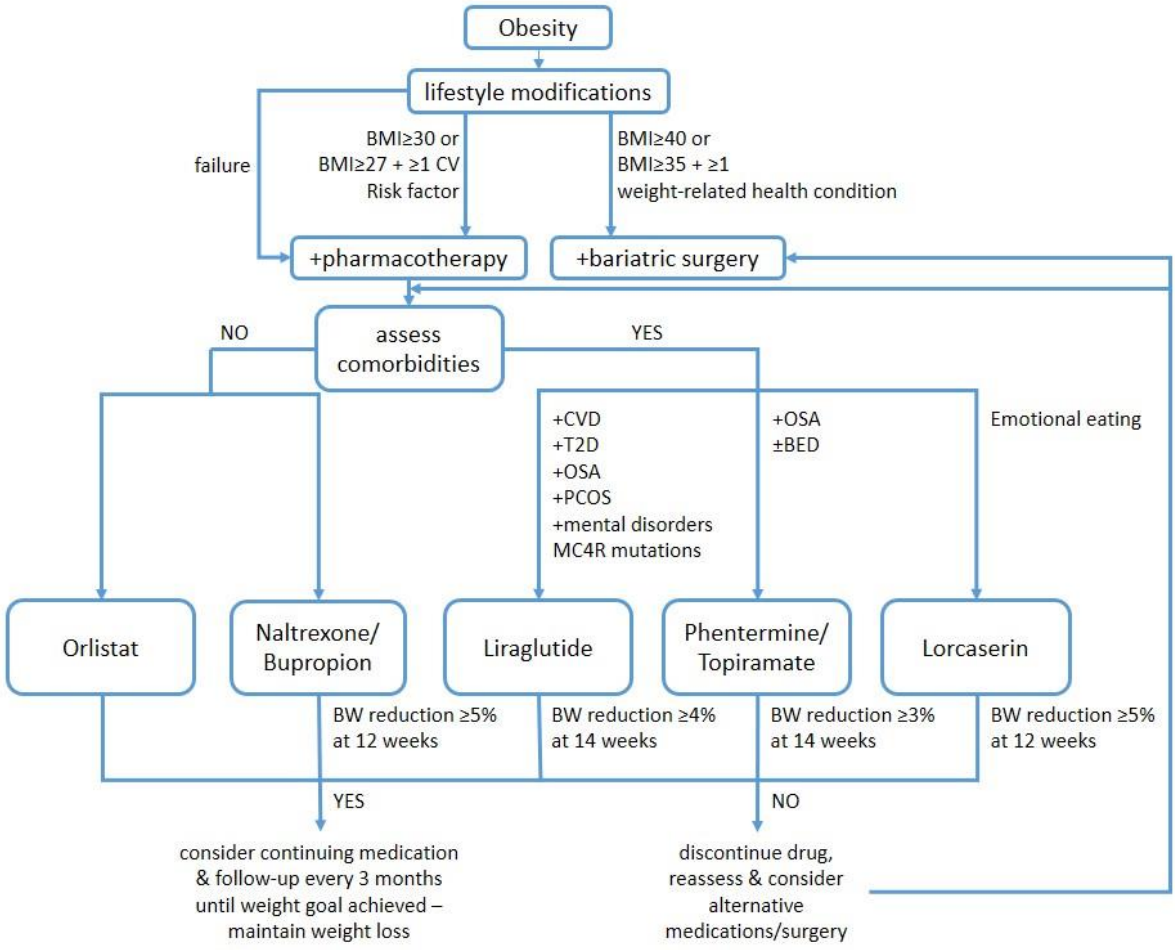

Figure 3. *Suggested flowchart outlining strategies for the ongoing management of obesity [18].*

1.1.4.1 Diet

Any dietary approach that creates a negative energy balance can effectively facilitate weight loss. However, maintaining weight loss is crucial, and success often relies on adhering to a restrictive dietary plan similar to that followed during the weight loss phase [19].

Recent reports on dietary plans for weight loss in overweight and obese adults, published within the last five years, point out several key findings highlighted in Table II. A balanced hypocaloric diet, ensuring adequate protein intake, has been shown to be effective for weight loss. While low-carbohydrate diets have demonstrated comparable weight loss outcomes to higher-carbohydrate isocaloric diets, the long-term safety of very low carbohydrate diets is uncertain. Various dietary approaches or specific dietary plans may also lead to weight loss by altering food intake and creating a negative energy balance, while potentially offering additional health benefits. However, recommendations for specific dietary regimens for long-term weight maintenance remain uncertain.

Table II. *Summary of recent research findings on dietary modifications for weight loss in overweight and obese adults, published after the 2013 AHA/ACC/TOC guidelines [19].*

Dietary intervention	Summary of findings
Very low carbohydrate diets	Higher weight loss in comparison of a moderately energy-restricted or low-fat diet.
Low carbohydrate diets	Comparable weight loss to that of an isocaloric diet with increased carbohydrate content
High protein diets	There is no consistent evidence that a high protein intake has a beneficial effect on weight loss and body composition.
Intermittent fasting/severe energy restriction	Has no additional weight loss benefits compared to continuous energy restriction
Meal replacements	Greater weight loss compared to conventional dietary plans.
Diets promoting specific food groups	Only minimal weight loss occurs without a defined energy restriction.
Diets closed to the Mediterranean dietary pattern	Weight reduction benefits only in the context of a hypocaloric diet. Evidence exists for other health-enhancing benefits.
Diets with varying energy distribution throughout the day	More significant weight loss observed with an early eating pattern..
Weight loss maintenance diets	There is insufficient evidence regarding the most suitable dietary approach for maintaining weight loss.

Popular dietary patterns such as plant-based diets and traditional or population-specific diets have gained attention for their potential in weight management. Among these, the Mediterranean dietary pattern stands out for its well-studied cardio-protective effects. Additionally, the timing of eating is recognized as significant in weight management [19].

Very low-calorie ketogenic diets (VLCKD) have emerged as successful nutritional strategies for managing obesity. These diets typically consist of 90% of calories from fat and 10% from carbohydrates and proteins. VLCKD offer several advantages, including rapid weight loss, which can foster a positive psychological cycle and enhance compliance with the diet. Moreover, VLCKD may help preserve fatty free mass, which plays a crucial role in glucose metabolism. It is recommended that VLCKD be followed with clinical support for a maximum of 12 weeks, either continuously or intermittently [20].

In 2016, VLCKD received strong endorsement from the Italian Society of Obesity (SIO) and the Italian Society of Endocrinology (SIE) for various conditions, including various types of obesity, management of extreme obesity before bariatric surgery, hypertension, hypertriglyceridemia, comorbidities that are not responsive to standardized diet protocols and childhood obesity with epilepsy or severe IR [20].

1.1.4.2 Exercise

Regular exercise plays a crucial role in weight management and metabolic health for individuals dealing with obesity. It contributes to weight and fat loss, helps in maintaining a healthy body weight, and improves overall metabolic fitness. Effective exercise programs should aim for a significant negative energy balance, long-term adherence, and positive effects on health.

When exercise is combined with dietary modifications, it can help sustain weight loss for up to 36 months. Increasing daily energy expenditure is essential in combating obesity, and this can be achieved through various types of exercise, including endurance or resistance training. The choice of exercise should be based on individual abilities, preferences, and responses. Exercise also has the potential to mitigate the chronic, low-grade inflammation associated with obesity. Additionally, improving metabolic fitness through exercise can counteract the adverse effects of obesity on CVD risk factors, ultimately reducing CVD mortality and IR [21].

1.1.4.3 Pharmacotherapy of obesity

Obesity is a complex and chronic condition, and its pharmacotherapy needs to address its main complications, such as T2DM, CVD, among others.

Specific anti-obesity pharmacotherapy targets various mechanisms:

- Decreasing appetite and caloric intake: Liraglutide, Lorcaserin
- Increasing energy expenditure: Bupropion
- Decreasing fat absorption: Orlistat, Naltrexone [18]

Metformin, typically used as the first-line agent for managing T2DM, is also employed for preventing weight gain in adults and children. In obese or overweight women with PCOS, metformin can reduce body weight by approximately 1 kg at 24 weeks, though it may not significantly affect BMI. In pregnant women with a BMI of 30 or higher, metformin has been shown to reduce maternal weight gain by approximately 1.4 kg without impacting new-born weight [22-24].

Anti-obesity vaccines, such as the anti-ghrelin vaccine, certain antiviral and genetic anti-obesity treatments that are based on circulating biomarkers, offer promising avenues for personalized pharmacotherapy, although they are still under investigation [25-27].

Recent preclinical and clinical reports have highlighted the role of gut microbiota in both preventing and treating obesity. Certain microorganisms, including Bifidobacterium, Enterococcus, Lactobacillus, Saccharomyces and Streptococcus, have shown the ability to attenuate immune responses related to chronic inflammation. Therefore, their supplementation in the diet could be important in obesity prevention or treatment. Probiotics, such as Lactobacillus and Bifidobacterium, have been found to reduce IR and increase satiety.

Additionally, augmenting dietary fibre intake with targeted prebiotics could potentially stimulate satiety hormones, thereby improving appetite regulation and assisting in weight control. Combinations of prebiotics with probiotics, known as “synbiotics” have shown promise in preventing obesity [28].

1.1.4.4 Bariatric surgery

When traditional methods like lifestyle changes and pharmacotherapy fall short, bariatric surgery emerges as the final recourse in managing obesity. By restructuring the digestive system, bariatric surgery offers a means to effectively reduce weight and mitigate obesity-related complications. Several types of bariatric surgery exist, including adjustable gastric band, biliopancreatic diversion with duodenal switch, and gastric bypass, sleeve gastrectomy each with its own set of advantages and drawbacks. Notably, bariatric surgery can enhance insulin sensitivity, regulate adipokine secretion, alleviate inflammation, and prompt beneficial cardio-metabolic alterations. Additionally, it influences various physiological factors such as gut microbiota, hormonal balance, and neural pathways involved in appetite and satiety regulation. However, it’s crucial to realize that bariatric surgery isn’t a panacea for obesity. It comes with limitations, including its irreversible nature in most cases, the need for lifelong monitoring and lifestyle adjustments, and potential complications like malnutrition, dumping syndrome, and surgical site infections [29-31].

1.2 Type 2 diabetes mellitus

1.2.1 Definition

T2DM is a chronic, highly heterogeneous, multifactorial disorder marked by both inherited and acquired IR, as well as qualitative and quantitative impairments in insulin secretion [32]. These abnormalities in insulin action and secretion lead to hyperglycaemia, a hallmark feature of diabetes mellitus. Chronic hyperglycaemia associated with diabetes mellitus is linked to complications affecting several organs, notably the heart, and circulatory system. eyes, nerves and kidneys.

1.2.2 Incidence

Diabetes represents a significant global public health challenge. According to the International Diabetes Federation (IDF), in 2017, approximately 451 million adults worldwide were reported to have diabetes, with the majority located in low- and middle-income nations. Without effective preventive measures, this figure is anticipated to rise to 693 million by 2045. (Figure 4.) [33, 34].

Diabetes ranks among the leading 10 causes of death globally. Alongside CVD, malignant tumours, and respiratory disease, these ailments collectively account for over 80% of premature deaths resulting from non-communicable diseases (NCDs). [33].

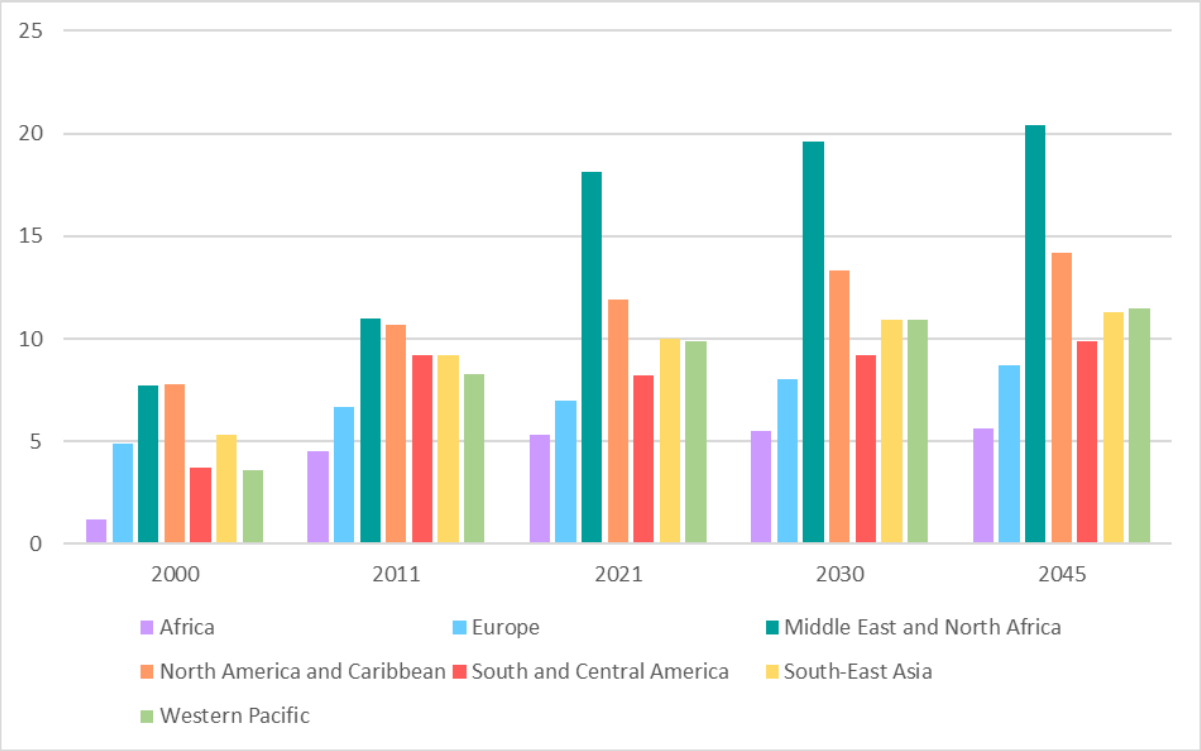

Figure 4. *Age-adjusted comparative prevalence of diabetes, %. Reproduced based on International Diabetes Federation World IDF Diabetes Atlas10th edition data [34].*

1.2.3 **Pathogenesis**

T2DM is a progressive condition characterized by distinct stages of development, influenced by genetic and environmental factors [35]. The stages of T2DM pathogenesis are presented in Figure 5.

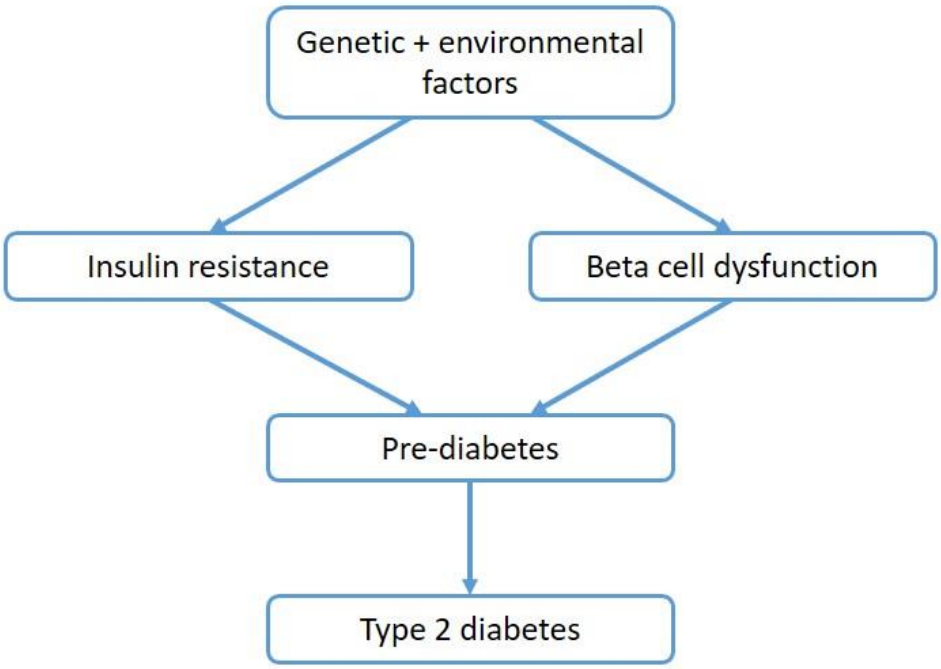

Figure 5. *Pathogenesis of T2DM [35]*

1.2.3.1 Genetic factors

Certain enzymes or protein factors implicated in insulin secretion and action are associated with the development of T2DM. The influence of hereditary factors outweighs that of environmental factors in secretory defects, while both genetic and environmental factors contribute equally to IR [35].

1.2.3.2 Environmental factors

The prevalence of T2DM rises with age, partly due to deteriorating glucose tolerance resulting from loss of muscle mass and increased adipose tissue (sarcopenia). This effect is particularly pronounced in sedentary individuals and further heightens IR in susceptible subjects [35].

Obesity stands out as the primary environmental contributor to the onset of T2DM and IR. The accumulation of visceral adipose tissue leads to elevated levels of free fatty acids (FFA), activating the β isoform of protein kinase C (PKCβ) and inhibiting the translocation of glucose transporter-4 (GLUT-4) to the cell membrane, thereby reducing glucose uptake into insulin responsive tissues [35]. TNFα and IL-6 disrupt proper insulin signalling by phosphorylating

IRS-1 on serine/threonine residues rather than tyrosine [35]. Adipose tissue releases various hormones known as adipocytokines, including leptin, adiponectin, and resistin, which can exacerbate IR, influencing certain pathologies associated with T2DM such as coronary diseases and dyslipidaemia.

Diet and nutrients are also important environmental factors implicated in the pathogenesis of T2DM. Excessive sugar consumption contributes to the development of T2DM and CVD [36]. Diets rich in mono-unsaturated and poly-unsaturated FA (for example Mediterranean diet) are linked to a reduced risk of T2DM [37]. Elevated fructose intake in the diet triggers the activation of peroxisome proliferator-activated receptor gamma (PPAR-γ), which contributes to lipogenesis, IR, and T2DM [38]. Conversely, a diet high in gluten content is linked to lower rates of T2DM due to increased cereal fibre intake, which promotes favourable alterations in gut microbiota [39].

Psychological stress represents a contributing factor to T2DM due to sympathetic activation, which impairs pancreatic β-cell function, thereby reducing insulin secretion. Simultaneously, in skeletal muscle, characterised by impaired insulin sensitivity and glucose uptake, furthermore reduced glycogen deposition, resulting in elevated blood glucose levels [35].

Depression, among various psychosocial factors associated with T2DM, has been extensively studied, revealing a bidirectional relationship between the two conditions. This relationship is bidirectional, meaning that depression can induce T2DM, and individuals with diabetes experience a 30% higher prevalence of depressive states compared to non-diabetic individuals [40].

Sleep disruptions, encompassing changes in duration and quality, respiratory function, hypoxemia, obstructive sleep apnoea, and circadian rhythm, have the potential to negatively impact glucose metabolism. Such disruptions lead to increased levels of growth hormone (GH), cortisol, inflammatory markers, and altered adipocyte function. Moreover, they result in decreased brain glucose utilization and elevated ghrelin levels, which contribute to obesity. Consequently, IR rises, and β-cell function declines, ultimately fostering hyperglycaemia and the onset T2DM. [41].

ROS, generated due to hyperglycaemia and elevated levels FFA, are believed to play a pivotal role in the initiation and advancement of T2DM. In pancreatic β-cells, ROS contribute to diminished insulin synthesis and secretion. Similarly, in peripheral tissues targeted by insulin, ROS promote the inactivation of insulin signal transduction [42].

In the context of T2DM pathogenesis, patients exhibit 11 distinct alterations [43-45]:

- Reduced insulin secretion in pancreatic β cells.

- IR in the liver, leading to increased gluconeogenesis
- Insulin resistance in muscular tissue, resulting in decreased glucose uptake
- IR in adipose tissue, leading to enhanced FFA production
- Decreased release of glucagon-like peptide 1 (GLP-1) in the gut, resulting in reduced incretin effect.
- Hyperglucagonemia due to increased production in pancreatic α cells
- Enhanced glucose reabsorption in the kidneys
- IR in the brain, with effects not fully understood.
- Alterations in gut microbiota composition
- Dysregulation of the immune system
- Increased glucose uptake in the gut

Initially, hyperinsulinemia can sustain normal fasting and postprandial glycaemia. This phase is characterized by elevated levels of FFA in obese individuals with IR [35]. Subsequently, during the prediabetic stages preceding the onset of T2DM, IR persists while the secretory capacity of pancreatic β cells declines, resulting in elevated fasting glycaemia and glucose intolerance. Chronic hyperglycaemia during these stages contributes to pancreatic β cell damage. Persistent hyperglycaemia, coupled with sustained IR, ultimately culminates in the clinical manifestation of diabetes [35].

1.2.4 **Complications**

Diabetes represents a chronic condition associated with complications that affect multiple organs (Figure 6). Microvascular complications encompass neuropathy, nephropathy, and retinopathy, impacting the nerves, kidneys, and eyes, respectively. Macrovascular complications involve CVD, stroke, and peripheral artery disease (PAD), impacting the heart, brain, and limbs, respectively. Diabetic foot syndrome, a significant cause of lower limb amputation, results from a combination of neuropathy, PAD, and infection. Other potential complications of diabetes include dental diseases, heightened susceptibility to infections, and pregnancy-related complications in women with gestational diabetes [46].

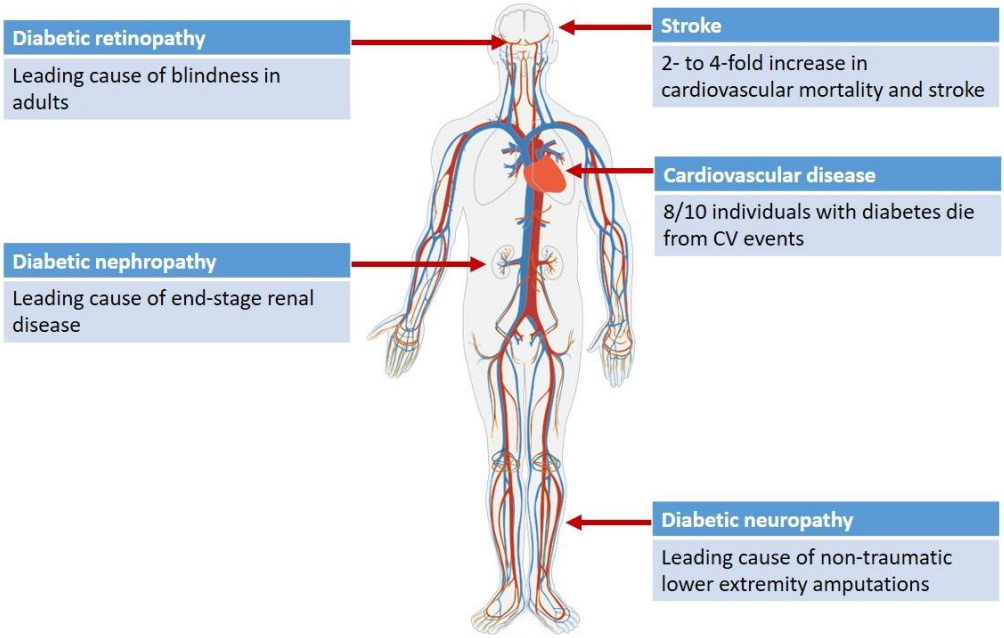

Figure 6. *Complications of T2DM [46]*

1.2.5 Therapeutic strategies of T2DM

1.2.5.1 Prophylactic therapy

There exists a correlation between excessive weight and the risk of developing T2DM, hypertension, and hyperlipidaemia. Dietary choices significantly influence both glycaemic parameters and body mass, making nutritional adjustments and consistent physical activity crucial for the prevention and management of T2DM.

Nutritional recommendations for patients with T2DM should be the followings:

- Avoidance of processed foods
- Reduction of sucrose intake to meet the WHO recommendation of <25 g/day
- Insulin-dependent patients should carefully consider the type and quantity of carbohydrates in each meal for optimal metabolic control
- Non-insulin-dependent patients should avoid foods that elicit spikes in blood glucose levels
- For individuals with T2DM and kidney insufficiency, is recommended a daily protein intake of 0.8 g/kg, while those with end-stage renal disease should increase protein intake to 1.2–1.3 g/kg
- Limiting consumption of sugars and sugar alcohols (e.g., sorbitol, xylitol)
- Avoiding frequent and abundant fatty foods consumption, opting instead for vegetable fats and dietary fibres [32]

The Mediterranean diet is advocated for managing weight and glycaemia in patients with T2DM. Substantial evidence supports the inverse correlation between adherence to the Mediterranean diet and the incidence of T2DM. Adherence to the Mediterranean diet may influence T2DM-related mechanisms, including anti-inflammatory/antioxidant actions, compounds that mimic glucagon-like peptides, and alterations in gut microbiota [47]. Currently, numerous leading researchers have validated that diets advocating limited carbohydrate intake lead to reductions in glucose and insulin concentrations, as well as body mass. One variant of a carbohydrate-restricted diet is the ketogenic diet, which is recommended for managing glycaemic variables in hyperinsulinemic obese patients with T2DM [48].

Consistent engagement in physical exercise, including resistance exercise, aerobic exercise, and fitness activities, can effectively improve glycaemic control by reducing IR and enhancing insulin secretion. Additionally, regular physical activity helps lower the risk of CVD and obesity among patients with T2DM [49]. Both aerobic and resistance training programs contribute to the development of healthier skeletal muscle, adipose tissue, liver, and pancreatic function in individuals with T2DM [50]. The exercise regimen recommended for patients with T2DM should be carefully tailored in terms of intensity and volume to maximize metabolic benefits while minimizing the risk of injury or CV complications [50].

1.2.5.2 Pharmacological therapy for T2DM

The primary objectives of treating patients with T2DM are twofold: to alleviate acute symptoms of hyperglycaemia and to prevent both macrovascular and microvascular complications [51].

Sulfonylureas (SUs), including Glibenclamide, Glimepiride, Glipizide, and Gliclazide, act by stimulating insulin secretion in pancreatic beta cells and reducing hepatic insulin clearance [51]. This class of medications is suitable for patients with preserved islet beta cell function but may be ineffective in those with impaired beta cell function. Prolonged use of SUs can potentially induce hypoglycaemia, making careful monitoring essential [52-54]. SUs are typically recommended as first-line pharmacotherapy in cases where metformin is contraindicated or not well tolerated [52-54]. However, they are associated with common adverse effects such as weight gain and hypoglycaemia [55]. Contraindications for SU use include hypersensitivity to sulfonylureas, pregnancy, and instances of reduced renal or hepatic function.

Meglitinides or glinides, including Meglitinide, Nateglinide, and Repaglinide, are secretagogues that elicit insulin release from beta cells, similar to SUs, although with differing chemical structures [51]. Meglitinides function by binding to the sulfonylurea receptor 1 (SUR1), although their affinity for SUR-1 is lower and they dissociate rapidly, resulting in a faster onset of action compared to SUs. They may serve as an alternative to SUs, particularly

in patients with irregular eating habits [56]. Despite their efficacy, meglitinides are associated with common side effects such as hypoglycaemia, respiratory tract infections, and headaches. Repaglinide, in particular, is contraindicated in individuals with severe liver dysfunction and is not recommended for use in patients over 75 years of age [57]. Additionally, meglitinides are contraindicated in type 1 diabetes mellitus (T1DM), diabetic ketoacidosis, and in individuals with severe liver dysfunction.

Biguanides (Metformin) enhance insulin sensitivity by augmenting peripheral glucose uptake, suppressing hepatic glucose production, and reducing glucose absorption in the gut [51, 58]. Therapeutically, Metformin is the primary pharmacological option for patients with T2DM who fail to achieve their glycated haemoglobin (HbA1c) target through lifestyle modifications alone. Intensive treatment with Metformin has been associated with significant reductions in diabetes-related mortality and myocardial infarction [51]. However, Metformin use is often associated with gastrointestinal adverse effects, particularly diarrhoea, and there have been warnings regarding the risk of lactic acidosis. Metformin is contraindicated in patients with moderate to severe renal impairment [51, 57, 58]. Furthermore, Metformin is contraindicated in individuals with congestive heart failure, advanced age (older than 80 years), and those with severe renal dysfunction [58].

Alpha-glucosidase inhibitors (AGIs), including Acarbose and Miglitol, function by inhibiting alpha-glucosidase, which leads to delayed intestinal digestion of complex carbohydrates and subsequent prolongation of postprandial glucose absorption [51]. Therapeutically, AGIs can serve as a first-line treatment for newly diagnosed T2DM patients who have not achieved adequate glycaemic control through diet and exercise alone. They can also be used in combination with other oral antidiabetic agents and insulin if monotherapy with these agents fails to meet HbA1c and postprandial blood glucose targets [59]. Common side effects of AGIs include gastrointestinal discomfort such as flatulence and diarrhoea [60]. AGIs are contraindicated in case of known hypersensitivity, inflammatory bowel disease such as , colonic ulcer, partial intestinal obstruction, predisposition to intestinal obstruction and diabetic ketoacidosis [60].

Thiazolidinediones (TZDs), including Pioglitazone and Rosiglitazone, exert their mechanism of action by reducing IR in target cells via transcriptional regulation of various genes implicated in glucose and lipid metabolism [51]. Therapeutically, due to the significant risk of adverse CV events associated with TZDs, such as heart failure, they are recommended by the American Diabetes Association as add-on therapy or monotherapy only if metformin is contraindicated [61]. Common side effects of TZDs include an increased risk of fatal and nonfatal heart failure,

bone fractures, weight gain, and oedema [62, 63]. TZDs are contraindicated in postmenopausal women, patients at high risk of fractures or history of osteoporosis, and patients taking other medications known to increase fracture risk, such as glucocorticoids and proton pump inhibitors (PPIs).

Glucagon-like peptide-1 (GLP-1) analogues, including Exenatide, Liraglutide, Dulaglutide, Lixisenatide, Semaglutide, Efpeglenatide, and Tirzepatide, act by enhancing postprandial glycaemic control through increased insulin secretion and decreased glucagon secretion from gut enteroendocrine cells. Additionally, they slow gastric emptying and reduce food intake, thereby optimizing nutrient absorption and limiting weight gain [64]. Therapeutically, GLP-1 analogues are recommended for the treatment of hyperglycaemia by increasing insulin secretion, repressing glucagon secretion and slowing gastric emptying to prevent postprandial glycaemic spikes, and promoting weight loss by reducing calorie intake [65]. The most notable side effect associated with GLP-1 analogues is gastrointestinal intolerance, manifesting as abdominal fullness, bloating, belching, flatulence, nausea, and vomiting. Another significant side effect, particularly observed with Exenatide, is an increased risk of pancreatitis [51]. Contraindications for GLP-1 analogues include patients with a history of pancreatitis.

DPP-4 (dipeptidyl peptidase 4) inhibitors, also known as gliptins, function by inhibiting the principal enzyme responsible for the degradation of endogenous GLP-1. By reducing the clearance of GLP-1, these inhibitors elevate concentrations of active GLP-1, leading to decreased fasting and postprandial glucose levels [66]. DPP-4 inhibitors are recommended as second- or third-line therapy for T2DM. They serve as a viable treatment option for patients without high-risk for CVD or heart failure (HF). DPP-4 inhibitors are particularly suitable for elderly patients with T2DM, in case of renal or hepatic diseases, and those at risk of hypoglycaemia [67-72]. Common adverse effects include headache, and hypersensitivity reactions The French Pharmacovigilance Database has indicated a heightened risk of bullous pemphigoid during exposure to DPP-4 inhibitors. Serious dermatological conditions such as Stevens-Johnson syndrome, as well as vasculitis, stomatitis, mouth ulceration, and hives have also been documented. Additionally, severe arthralgia and disabling joint pain may occur. There is a potential risk of worsening renal impairment, particularly in older patients or those with high CV or renal risk factors, including pre-existing chronic kidney disease [66, 73, 74]. Contraindications for DPP-4 inhibitors include patients with a history of pancreatitis, hypoglycaemia, and angioedema [74].

Sodium-glucose cotransporter-2 inhibitors (SGLT2i), including Canagliflozin, Dapagliflozin, Empagliflozin, and Ertugliflozin, exert their therapeutic effects by inhibiting renal glucose

reabsorption. This inhibition occurs through the blockade of SGLT2 cotransporters located in the proximal tubules, leading to glycosuria and subsequent reduction in glycaemia and HbA1c levels [75]. The nephroprotective effects of SGLT2i is attributed to the excretion of sodium which provide an improved tubuloglomerular feedback accompanied by a decrease of the intraglomerular pressure [76]. Moreover, glycosuria leads to caloric loss, which in turn contributes to weight reduction, and improves insulin sensitivity and lipid metabolism. This process supposedly alleviates lipotoxicity as well. Furthermore, evidence suggests that SGLT2i reduce glucotoxicity in the tubular cells by alleviating mitochondrial dysfunction and inflammation [77]. These inhibitors also potentially diminish kidney hypoxia by lowering tubular energy and oxygen demand [75, 78]. Improvement in blood pressure results from negative sodium and water balance and possibly inhibition of the sympathetic nervous system, contributing to enhanced CV function and the CV benefits associated with SGLT2i [79]. Additionally, SGLT2 inhibitors decrease hepcidin levels, thereby enhancing erythropoiesis and ameliorating anaemia [80]. Furthermore, potential mechanisms of action include reductions in inflammatory markers, fibrosis, and other related pathways [78, 81-83].

Therapeutic recommendations for Sodium-Glucose Cotransporter-2 Inhibitors encompass several key aspects:

- Improving glycaemic control in T2DM when used adjunctively with diet and exercise [84].
- As adverse effects reducing major CV events, including non-fatal myocardial infarction, non-fatal stroke, and CV death, particularly in patients with T2DM mellitus and CVD [85].
- In patients with chronic kidney disease at risk of progression lowering the risk of estimated glomerular filtration rate (eGFR) decline and subsequent hospitalization [86].
- Managing obesity, often in combination with glucagon-like peptide -1 receptor agonists [87].
- Treating Non-alcoholic Fatty Liver Disease (NAFLD) by serving as auxiliary therapy in patients with T2DM and NAFLD [88].

Common side effects associated with SGLT2 inhibitors include female genital mycotic infections, urinary tract infections, increased urination, nausea, and constipation. Additionally, there are risks of lower limb amputation, diabetic ketoacidosis, acute kidney injury, hypoglycaemia, allergic reactions, as well as hyperkalaemia and dyslipidaemia [89]. The development of anaphylaxis or angioedema to any of the four agents serves as an absolute contraindication [90].

Insulin serves as a specific therapy for T1DM and is also administered in cases of T2DM when oral antidiabetic medications or various injectable agents fail to stabilize blood glucose levels. It is produced using recombinant DNA technology [51].

Combinations of insulin with metformin, DPP-4 inhibitors, or GLP-1 agonists can offer sustained glycaemic control over extended periods. The availability of various insulin formulations and administration aids allows for tailored therapy [57].

Insulin exerts its effects by binding directly to its receptors on cell plasma membranes. These receptors are distributed across various cell types, with the highest density found on hepatocytes and adipocytes. The insulin receptor is a heterotetrameric glycoprotein comprising alpha and beta subunits. The extracellular alpha subunits express the insulin-binding sites, while the transmembrane beta subunits have tyrosine kinase activity [91].

Upon insulin binding to the alpha subunits, it activates tyrosine kinase activity within the beta subunits, leading to the translocation of glucose transporters from the cytoplasm to the cell surface [92]. Consequently, these glucose transporters facilitate the uptake of glucose from the bloodstream into the cell, thereby lowering blood glucose levels [93].

Insulin is categorized based on its onset, peak time, and duration into ultra-rapid acting, rapid-acting, intermediate, long-acting, and ultra-long-acting types. Additionally, it can be classified according to its origin into analogues and human insulin [94, 95] (Table III.).

Table III. *Types of insulin*

Types of insulin	Class	Start of action	Max peak	Duration
Ultra-rapid acting	Analogues			
Faster aspart (Fiasp)		within 5 minutes	30–60 minutes	3–4 hours
URLi (insulin lispro-aabc)		within 5 minutes	30–60 minutes	3–4 hours
Rapid acting	Analogues			
Lispro, Aspart, Glulisine		5–15 minutes	30–90 minutes	3–5 hours
Afrezza (inhaled)		3–7 minutes	12–15 minutes	1½–4½ hours
Short-acting (Regular)	Human	30–60 minutes	2–4 hours	6–8 hours
Intermediate (NPH)	Human	2–4 hours	6–10 hours	12–18 hours
Long-acting	Analogues			
Glargine U100		2–4 hours	8–12 hours	22–24 hours
Detemir		1–2 hours	4–7 hours	20–24 hours
Ultra Long- acting	Analogues			
Degludec		1½–2 hours	Minimal/no peak	> 42 hours
Glargine U300		2–6 hours	Minimal/no peak	30–36 hours
Pre-mixed insulin				
biphasic insulin aspart 70/30				
Novo Mix™ 30		10–20 minutes	1–4 hour	24 hours
insulin lispro mix 25				
Humalog™ Mix25™		15–30 minutes	30–70 minutes	24 hours
insulin lispro mix 50				
Humalog™ Mix50™		15–30 minutes	30–70 minutes	24 hours
Humulin 70/30		30–60 minutes	1–5 hours	24 hours

The recommended classes of hypoglycaemic agents for treating T2DM and correcting various metabolic disorders are illustrated in Figure 7.

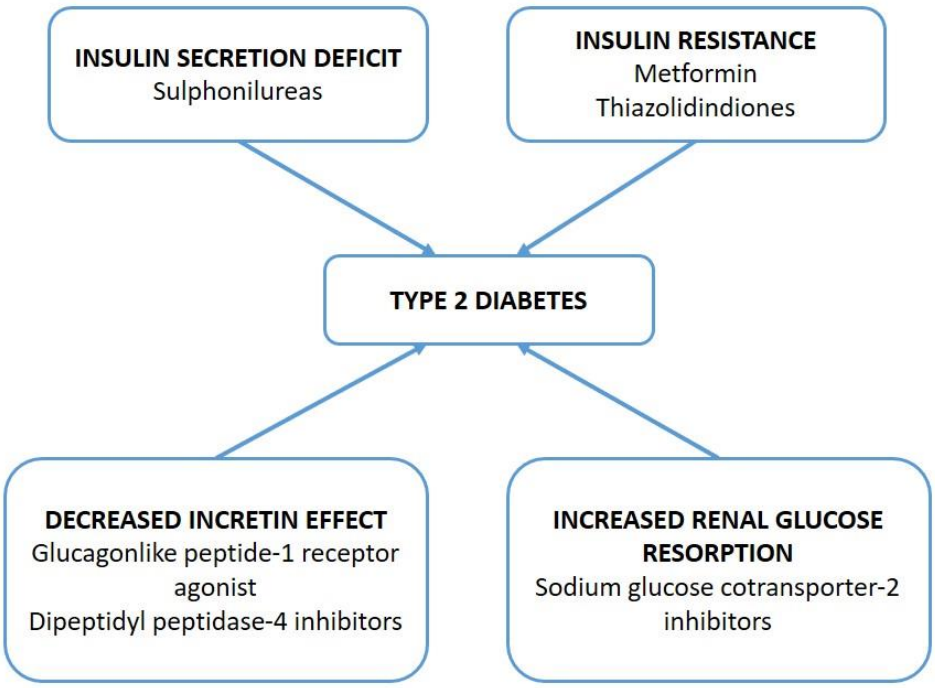

Figure 7. *Pathological changes associated with T2DM and its pharmacological treatment. [96]*

New directions in the treatment of T2DM have focused on exploring the mechanisms underlying alterations in gut microbiota. The gut microbiota comprises bacteria, yeast, and viruses, with dominant phyla including Firmicutes (e.g., Lactobacillus, Bacillus, Clostridium, Enterococcus, and Ruminococcus), Bacteroidetes (e.g., Bacteroides and Prevotella), Actinobacteria (e.g., Bifidobacterium), Proteobacteria, Fusobacteria, and Verrucomicrobia [97]. In a study investigating the potential influence of altered gut microbiota on T2DM development, researchers collected samples of Bacteroides, Proteobacteria, Firmicutes, and Actinobacteria from both T2DM patients and healthy controls. Their findings revealed significant variations in the intestinal flora composition among T2DM patients, characterized by a reduction in Lactobacilli spp. and F. prausnitzii. This alteration was associated with IR [98].

Among antidiabetic agents, Metformin, a primary pharmacological agent for the treatment of T2DM, is known to ameliorate intestinal bacterial dysbiosis. Additionally, herbal remedies can be integrated with conventional pharmacotherapy to address intestinal bacterial dysbiosis and regulate glycaemic levels. Among these botanical interventions, Momordica charantia stands out as one of the most extensively studied, therapeutically utilized, and widely accepted hypoglycaemic plants [99, 100]. Momordica charantia, commonly known as Bitter Melon, is

indigenous to tropical and subtropical regions. It has been utilized extensively in traditional medicinal practices such as Chinese medicine, Hindu medicine, and Ayurveda. The diverse array of bioactive compounds found in Momordica charantia, including polysaccharides, proteins, and peptides, as well as peroxidase, saponins, terpenoids, flavonoids and phenolic compounds, alongside isorhamnetin, predominantly found in its fruits and seeds, underlie its therapeutic properties, which include antioxidant, antidiabetic, hepatoprotective, antibacterial, and anti-inflammatory effects [101-103]. Due to its complex nutritional composition, which includes vitamins, minerals and fibres, Momordica charantia holds potential for preventing metabolic diseases, including T2DM [99, 101]. As a well-established antidiabetic agent, Momordica charantia facilitates insulin secretion, enhances glucose uptake by insulin sensitive tissues, and impedes glucose absorption from the intestines while suppressing glucose production from the liver [99, 104]. However, caution is warranted in administering Momordica charantia in individuals with glucose-6-phosphate dehydrogenase deficiency, liver diseases, during pregnancy, and in small children [105].

1.3 Diabetic cardiomyopathy

1.3.1 Definition of diabetic cardiomyopathy

Diabetic Cardiomyopathy (DCM) is a pathophysiological condition induced by DM that can culminate in heart failure [106]. First identified in diabetic patients by Shirley Rubler in 1972 [107], currently the European Society of Cardiology (ESC) characterizes cardiomyopathy as a heart muscle disorder typified by structural and functional abnormalities in the myocardium, independent of coronary artery disease (CAD), valvular, hypertensive, or congenital heart ailments [108]. This pathology is believed to arise from diffuse myocardial fibrosis, cardiac hypertrophy, and diabetic microangiopathy [106].

DCM typically manifests initially with myocardial fibrosis, hypertrophy, and cardiac stiffness alongside diastolic dysfunction, potentially progressing to subsequent systolic dysfunction and ultimately leading to clinical HF [109]. Clinically, DCM presents with cardiac hypertrophy and diastolic dysfunction, potentially resulting in heart failure with preserved ejection fraction (HFpEF) [110].

1.3.2 Incidence and prevalence of diabetic cardiomyopathy

Diabetes mellitus poses a significant risk for CVD, with T2DM notably associated with an elevated risk of CV complications [111, 112]. The Framingham Heart Study, conducted in 1974, was one of the pioneering studies to establish this link, reporting an incidence of CVD 2.4- and 5-fold higher, respectively, in both men and women with diabetes after adjusting for common CVD risk factors [113]. Atherosclerotic diseases have traditionally been considered the predominant manifestations of CVD in T2DM. Notably, patients with diabetes mellitus have up to a 74% increased risk of developing heart failure, and those with both DM and HF face a four-fold greater mortality risk compared to those without HF. Research indicates a higher incidence of heart failure among diabetic (39%) versus non-diabetic (23%) individuals, with a relative risk of 1.3 for HF development after 43 months of observation [114, 115].

DCM is relatively prevalent in the community, with an estimated prevalence of 1.1%. The prevalence of DCM is on the rise in tandem with the increasing prevalence and severity of diabetes mellitus. In T1DM and T2DM, each 1% increase in HbA1c levels was associated with a 30% and 8% increase in the risk of heart failure, respectively, independent of other risk factors including smoking, obesity, dyslipidaemia, hypertension, and coronary heart disease. This suggests that incremental increases in blood glucose levels significantly contribute to heart

failure in diabetic patients. Furthermore, the prevalence of HF escalates notably with advancing age [115-117].

CV risk tends to be higher in diabetic women compared to diabetic men, possibly attributable to sex hormones and neurohormonal diversity, alongside gender-specific activation of molecular pathways implicated in cardiac metabolism and remodelling [118]. Findings from the Framingham Heart Study revealed an increased incidence of HF in both male and female diabetic patients compared to age-matched counterparts, with this association remaining independent of metabolic and CV factors [113, 119, 120].

1.3.3 Pathogenesis of diabetic cardiomyopathy

The pathogenesis of DCM remains incompletely elucidated, characterized by multifactorial origins. Proposed mechanisms encompass metabolic disorders, IR, dysregulation of the renin-angiotensin system (RAS), autonomic dysfunction in the myocardium, microvascular complications, and myocardial fibrosis.

Chronic hyperglycaemia is believed to hold a pivotal role in DCM development, although the pathogenesis involves intricate molecular and metabolic events in the heart and systemic circulation. Hyperglycaemia and systemic, as well as cardiac IR, are independently linked to cardiac dysfunction and HF in DM [121-123]. Elevated blood glucose levels and glucotoxicity can trigger protein glycation reactions, leading to the formation of advanced glycation end products (AGEs). These AGEs arise from non-enzymatic glycosylation of amino acids, lipoproteins, and lipids. AGEs modify the mechanical properties of the extracellular matrix by enhancing resistance to enzymatic proteolysis of connective tissue and promoting the interlinking of collagen and laminin. This process encourages fibrosis, decreases cardiac compliance, and causes left ventricular diastolic dysfunction [124]. Additionally, AGEs are implicated in escalating the generation of ROS, which foster inflammation and fibrosis [123]. Consequently, hyperglycaemia, glucotoxicity, and the associated activation of AGEs/receptor for AGEs (RAGE) signalling play significant roles in the genesis of myocardial fibrosis, a critical component of diabetic cardiomyopathy [109]. A notable elevation in AGEs and their interaction with cell surface receptors known as RAGEs initiates a series of pathophysiological responses that contribute to severe cardiac injury. Among these responses, the activation of protein kinase C (PKC) and NADPH oxidase (NOX) pathways facilitates the generation of peroxides and ROS. This process culminates in the maladaptive activation of mitogen-activated protein kinase (MAPK) and NF-κB signalling cascades, resulting in the production of various inflammatory and pro-fibrotic factors. Additionally, there is an upregulation of apoptosis

mediated by p53 and calcineurin signalling, along with autophagy [125-128]. Collectively, these mechanisms can induce functional and structural impairment, culminating in cardiomyocyte death and eccentric left ventricular remodelling accompanied by systolic dysfunction.

Furthermore, hyperglycaemia serve as a crucial trigger for the induction of chemokines, cytokines, and leukocyte adhesion molecules, culminating in myocardial inflammation. This inflammatory process within the myocardium is multifaceted, involving various pathways implicated in the pathogenesis of diabetic cardiomyopathy. Chronic low-grade inflammation plays a pivotal role in mediating structural and metabolic alterations in the diabetic heart, including left ventricular hypertrophy (LVH), myocardial fibrosis, and perturbations in calcium handling [115]. Additionally, persistent systemic inflammation in diabetes triggers leukocyte activation and migration to multiple organs, promoting inflammatory tissue remodelling and eventual fibrosis. Within the heart, this inflammatory cascade can reduce cardiac output, thereby exacerbating cardiac inflammation and fibrosis, ultimately culminating in myocardial dilation and HF [129].

Various stimuli associated with DCM, such as hyperlipidemia, hyperglycaemia, and hyperinsulinemia, trigger inflammatory signalling pathways, including nuclear factor-kappa B (NF-κB), toll-like receptors (TLRs), inflammasome activation, and pyroptosis. Micro-RNAs (miRNAs) and long noncoding RNAs (lncRNAs) have emerged as crucial regulators of inflammatory signalling pathways in diabetic hearts [130]. Subsequent to the activation of NF-κB, detrimental inflammatory cascades occur within the myocardium of individuals with DM, leading to cardiomyocyte hypertrophy, fibrosis, apoptosis, and impairment of myocardial energetics and contractility (Figure 8.) [131]. Additionally, NF-κB governs DNA transcription and promotes the expression of pro-inflammatory cytokines, along with the assembly of the NLR family pyrin domain-containing 3 (NLRP3) inflammasome. Activation of the NLRP3 inflammasome is pivotal in the pathogenesis of HF in diabetes. Upregulation of NLRP 3 facilitates the myocardial infiltration of inflammatory cells. Conversely, downregulation of NLRP3 attenuates cardiomyopathy in an experimental model of T2DM in rats [109, 132].

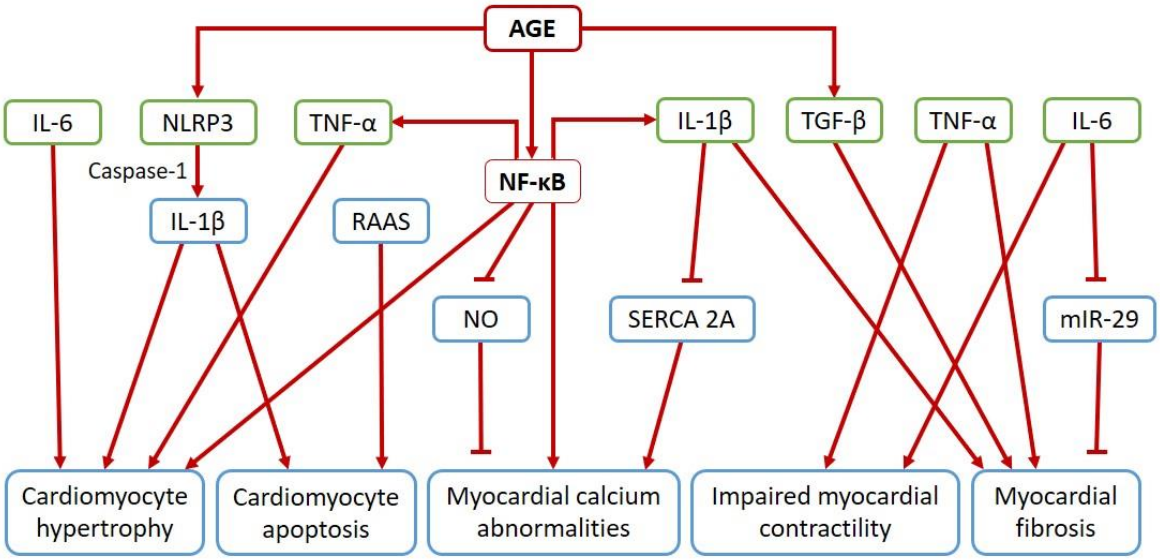

Figure 8. *Inflammatory pathways implicated in the progression of pathological cardiac remodelling and dysfunction observed in DCM [115].*

Lipotoxicity plays a significant role in the pathogenesis of DCM by impairing physiological autophagy in cardiomyocytes and promoting apoptosis. Increased plasma FFA levels lead to heightened myocardial FFA uptake and subsequent intra-myocardial lipid accumulation, highlighting the regulatory role of serum FFAs in myocardial lipid deposition [133]. In individuals T2DM, upregulated expression of lipoprotein lipase (LPL) on cardiomyocytes enhances the hydrolysis of VLDL in the coronary circulatory system, augmenting lipid availability [134]. Consequently, cardiomyocyte lipid uptake is elevated, potentially promoting fatty acid oxidation, which is probable exacerbated by reduced glucose uptake due to IR and repression of glucose oxidation by certain lipid catabolism intermediates (Acetyl-CoA and citrate) [135]. Various lipid metabolites, including diacylglycerols (DAGs) and ceramides, play a pivotal role in impairing insulin signalling, thereby exacerbating DCM. Elevated levels of DAG within cardiomyocytes activate various isoforms of protein kinase C (PKC), leading to diminished insulin signalling and nitric oxide (NO) production. Ceramide, on the other hand, can directly activate atypical PKCs while inhibiting insulin Akt/PKB signalling, thereby impairing GLUT-4 translocation and insulin-stimulated glucose uptake in diabetic cardiac tissues [115, 136]. Consequently, the accumulation of lipids and lipid metabolites in the cardiomyocytes contributes to cardiac IR, decreased bioavailability of NO, cardiac inflammation and fibrosis, culminated with diastolic dysfunction [109].

Cell apoptosis induced by lipotoxicity, often termed lipoapoptosis, encompasses various mechanisms including ER stress, ceramide and DAG accumulation, palmitate toxicity,

membrane destabilization, and inflammation [137]. Lipoapoptosis contributes to structural damage and myocardial fibrosis, ultimately compromising cardiac function.

Oxidative stress is a significant contributor to the pathogenesis of DCM. It promotes cardiac fibrosis and hypertrophy. Diabetes exacerbates oxidative stress through several mechanisms: dysregulation of the electron transport chain in mitochondria; upregulation of the RAS and NOX activity; and accumulation of AGEs [138, 139]. Oxidative stress and inflammation synergistically enhance the production of ROS and inflammatory mediators, thereby promoting and worsening remodelling and cardiac dysfunction.

Mitochondrial dysfunction is central to the pathogenesis of diabetic cardiomyopathy and the ensuing heart failure. At the level of myocardial tissue of diabetic individuals, metabolic and oxidative stresses heighten the sensitivity of the mitochondrial permeability transition pore to calcium ions, consequently, leading to cardiomyocyte autophagy and cardiac necrosis [119].

In conclusion, the pathophysiological process of DCM involves multiple factors, including oxidative stress, mitochondrial dysfunction, elevated formation and deposition of AGEs, impaired handling and function of mitochondrial calcium ions, inflammation, activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS), ER stress, microvascular dysfunction, and disorders in cardiac metabolism (Figure 9) [109, 119]. The molecular mechanisms underlying these pathophysiological changes encompass abnormalities in various cellular pathways, including those involving AMP-activated protein kinase (AMPK), PPARs, O-linked N-acetylglucosamine (O-GlcNAc), PKC, miRNA, and exosomes [115]. Furthermore, a variety of proteins and signalling pathways, including AMPK, PPARs, O-GlcNAc, SGLT2, PKC, MAPK, NFκB, Nrf2, SIRT1, miRNA, and exosomes, are implicated in these underlying pathophysiological events [115, 138].

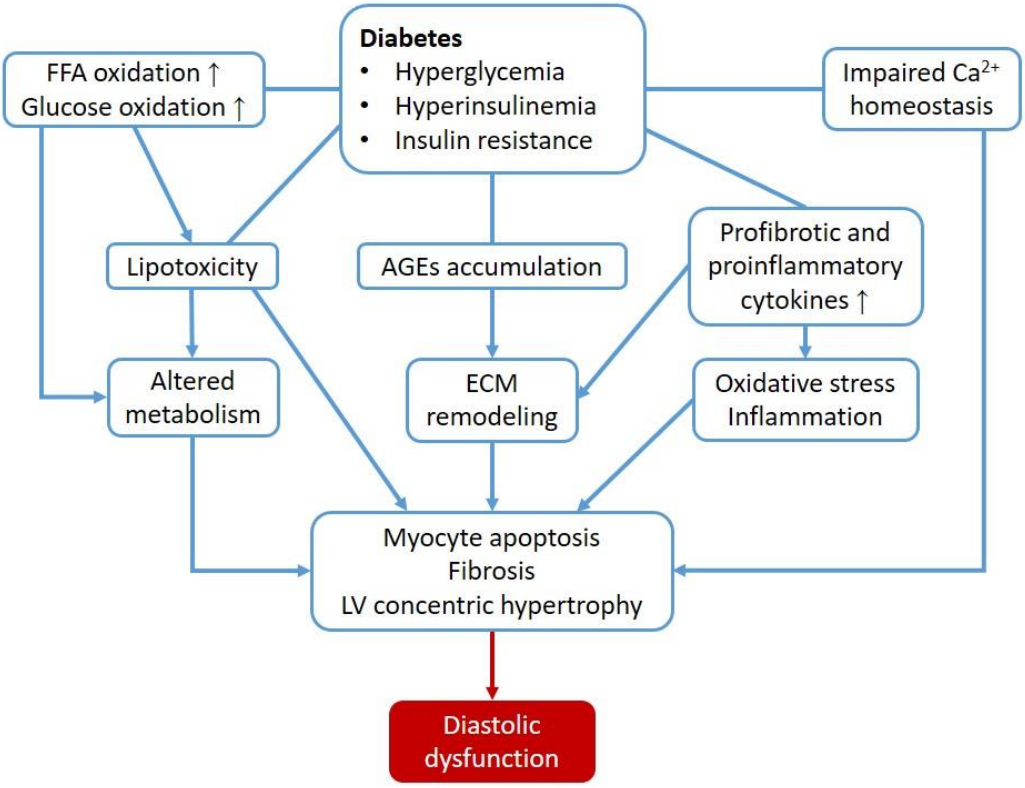

Figure 9. *Pathophysiological mechanisms in DCM [119]*

Clinically, the diabetic heart exhibits diastolic dysfunction characterized by preserved ejection fraction. These changes stem from pathological remodelling of the heart, marked by augmented interstitial and perivascular fibrosis, along with left ventricular hypertrophy [119]. The progression of DCM manifests in three discernible stages exhibiting unique pathophysiological characteristics and clinical implications. In the initial stage, IR and hyperglycaemia, occur without significant alterations in myocardial structure or systolic function. During the advanced stage of DCM, cellular alterations such as impaired autophagy of apoptotic or necrotic cells, oxidative stress, and dysregulated immune response contribute to extended cardiac fibrosis. These changes precipitate significant modifications initially in diastolic function, followed later by alterations in systolic function.

In the final stage of DCM, metabolic alterations, neurohumoral activation, and myocardial fibrosis exacerbate impairment in coronary microcirculation, diastolic and systolic function. Structural anomalies characteristic of DCM encompass gradual disappearance of myofibrils, cardiomyocyte necrosis, collagen deposition in connective tissue, interstitial fibrosis, hypertrophy, sclerosis of small coronary vessels, thickening of basement membranes, hyaline arteriolar sclerosis, and capillary microaneurysms [123].

1.3.4 Prophylactic and medical therapy of diabetic cardiomyopathy

Recent advancements in understanding the pathogenesis and pathophysiology of DCM have led to improved management strategies for affected patients. These encompass lifestyle modifications, optimized diabetic control, lipid-lowering therapy, and the treatment of concurrent conditions like hypertension and CAD. Lifestyle changes such as regular physical exercise and dietary adjustments to manage caloric intake are pivotal in preventing obesity and reducing the incidence of DM and its associated cardiac complications, including DCM.

Prophylactic measures to prevent DCM complications include lifestyle modifications promoting exercise, maintaining a balanced caloric intake, weight management, smoking cessation, as well as the use of anti-diabetic medications and lipid-lowering therapies. Effective management of CV risk factors, particularly glycaemic control, can delay the onset and progression of cardiac complications in diabetic individuals [119, 122].

Administration of antioxidant dietary supplements such as Coenzyme Q10, Vitamin E, Zinc, Resveratrol, and Sulforaphane has shown promise in reducing ROS accumulation, thereby mitigating cardiac inflammation, fibrosis, and hypertrophy in both T1DM and T2DM. Modulating oxidative stress represents a preventive and therapeutic approach for managing diabetic cardiomyopathy [115, 140].

The therapeutic approach for diabetic cardiomyopathy (DCM) revolves around managing the underlying diabetes, mitigating cardiotoxicity, and addressing the risk factors associated with CVD progression [141].

1.3.4.1 Antidiabetic therapy

Improved glycaemic control has been linked to favourable outcomes in diabetic microvascular complications in numerous clinical trials [122]. Certain antidiabetic agents play a role in the prevention and treatment of DCM and associated CVD in patients with T2DM. These include sulfonylureas, biguanides, sodium-glucose cotransporter-2 (SGLT-2) inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists, dipeptidyl peptidase 4 inhibitors, peroxisome proliferator-activated receptor (PPAR) agonists, and insulin.

Sulfonylureas stimulate the release of insulin from islet β cells without impacting endogenous insulin synthesis. This drug class is suitable for patients with preserved islet beta cell function but is ineffective for those with impaired function. However, prolonged use of sulfonylureas can lead to hypoglycaemia. Additionally, there is evidence suggesting that sulfonylureas may elevate the risk of heart failure. A retrospective study compared the incidence of myocardial

infarction, overall mortality, and heart failure development among various diabetes treatment groups [142-144].

Metformin, improves diabetic cardiomyopathy (DCM) by modulating glycolipid metabolism, decreasing advanced glycation end product (AGE) formation, suppressing the NLRP3 inflammasome, and enhancing mitochondrial function, thereby exerting cardioprotective effects [145].

SGLT-2 inhibitors have demonstrated reductions in HbA1c levels, blood pressure, weight, visceral adiposity, and oxidative stress in T2DM patients. SGLT2i medications can impede glucose reabsorption, significantly impacting cardiac function in DCM models, thus presenting a novel and promising therapeutic avenue for DCM. SGLT2i induces natriuresis, osmotic diuresis, plasma volume diminution, and decrease of blood pressure and arterial stiffness, mechanisms potentially alleviating DCM and GF. Additionally, the cellular metabolism can be shifted from glucose to FA oxidation. Consequently, the beneficial cardiac effects of SGLT2 may involve increased utilization of the most efficient substrate for cardiac metabolism, namely ketone body β-hydroxybutyrate. SGLT2i has been demonstrated to enhance CV outcomes and reduce mortality in individuals with T2DM. First drugs targeting SGLT-2, such as empagliflozin and dapagliflozin, have exhibited protective effects on cardiac function in diabetic models, with dapagliflozin eliciting regression in increased left ventricular mass in patients with T2DM [109, 115, 146-152].

GLP-1 has received substantial attention as a promising therapeutic method for diabetes mellitus due to its capacity to promote insulin secretion. Prior investigations have revealed that both GLP-1 and its analogues confer protection to the heart against ischemia-reperfusion injury and diabetic complications. A study outlined that the cardioprotective effects of GLP-1, encompassing reductions in lipid accumulation and enhancement of antioxidant and anti-apoptotic properties, may be mediated through a mechanism involving PPARα [153, 154]. GLP-1 receptor agonist (GLP-1RA) exhibit the capability to augment myocardial insulin sensitivity, enhance glucose uptake rates, strengthen myocardial energy metabolism, and supress cardiomyocyte apoptosis, thereby contributing to the management of DCM. A newly developed GLP-1RA, namely Oral Hypoglycaemic Peptide 2 (OHP2), has shown efficacy in mitigating DCM in rat models induced by high-fat diet (HFD) and continuous streptozotocin (STZ) injection. OHP2 treatment led to reductions in both hyperlipidemia and myocardial lipid accumulation. Moreover, OHP2 administration reversed oxidative stress and mitigated mitochondrial dysfunction in diabetic hearts [154-157].

DPP-4 activity extends beyond glucose metabolism, encompassing regulation of diverse processes such as inflammation, vascular function, cell survival. DPP-4 inhibitors have demonstrated potential in averting cardiac dysfunction by the inhibition of the Nlrp3/ASC pathway. However, there exists limited evidence supporting a preventive role for DPP-4 inhibitors in heart failure. Clinical investigations have indicated that DPP-4 inhibitors do not ameliorate LV diastolic function in diabetic patients. Surprisingly, large-scale outcome trials have unveiled heterogeneous and partly adverse (or null) effects of DPP-4 inhibitors on CV health [141, 158, 159].

TZDs serve as insulin sensitizers, exerting their effects on intracellular metabolic pathways. In this regard TZDs augment insulin action and enhance insulin sensitivity in certain tissues. Additionally, TZDs elevate adiponectin levels, diminish hepatic gluconeogenesis, and boost insulin-dependent glucose uptake in muscle and adipose tissues. TZDs modulate gene expression by binding to the nuclear transcription regulator PPAR-γ. PPAR-γ agonists ameliorate IR by upregulating adiponectin and GLUT4 expression while counteracting the effects of TNF-alpha in adipocytes. Pioglitazone, a TZD, is frequently utilized for secondary prevention owing to its robust efficacy in averting CV events. To mitigate the risk of heart failure onset due to fluid retention, combination therapy with a mineralocorticoid receptor antagonist or thiazide diuretics is recommended alongside pioglitazone [139, 160, 161].

Correction of hyperglycaemia through insulin therapy has been shown to mitigate cardiomyocyte hypertrophy, reduce collagen deposition, alleviate diastolic dysfunction, and impede the advancement of diabetic cardiomyopathy [109].

Attaining optimal long-term glycaemic control with a solitary anti-diabetic agent is infrequently achievable in patients diagnosed with T2DM. Consequently, the selection of appropriate combination anti-diabetic therapy should be tailored to the individual's clinical circumstances for the management of patients with DCM.

1.3.4.2 Cardio-vascular therapy

Various commonly prescribed drugs exhibit beneficial effects on the damaged heart in DCM:

Statins have demonstrated efficacy in downregulating vascular eNOS expression, attenuating ROS synthesis, enhancing LV function, and mitigating myocardial fibrosis to prevent DCM [162, 163]. Numerous prior investigations have outlined the effectiveness of statins in preventing major cardiac and cerebrovascular events, attributed to their so-called "pleiotropic" actions, encompassing anti-inflammatory, antithrombotic, anti-atherosclerotic, antiproliferative, and antioxidative properties. Given that lipophilic statins may elevate blood glucose levels and exacerbate diabetes severity, glucose monitoring is advisable during statin

therapy in DCM patients. Hydrophilic statins, such as pravastatin and fluvastatin, do not compromise glycaemic [164-166].

RAAS blockers have been found to lower blood pressure, reduce IR, and enhance myocardial diastolic function in DCM [167, 168]. Although the incidence and mortality rates associated with CVD in diabetic patients receiving angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs) have shown a decrease, the effect appears to be modest [169].

ACEIs exert broad effects on microvascular and macrovascular complications in DM and may improve myocardial fibrosis by modulating the actions of angiotensin II. Additionally, evidence suggests a favourable effect of aldosterone antagonism in diastolic heart failure, attributed to their beneficial effects on cardiac hypertrophy and fibrosis [162]. Spironolactone, an aldosterone antagonist, has been demonstrated to inhibit oxidative stress response in diabetic mice by increasing the antioxidant activity of glutathione peroxidase and catalase, thereby improving cardiac function [170, 171].

Beta-receptor antagonists have been shown to enhance cardiac function in DCM by reducing cardiomyocyte glucose uptake, elevating levels of oxidative stress enzymes such as SOD1 and SOD2, and inhibiting the production of ROS [172, 173]. Experimental models of DCM have underscored the effectiveness of beta-receptor blockers. Given the established benefits of beta-blockers in HF, this class of drugs should be taken into account for DCM treatment. Additionally, they can serve as effective antihypertensive agents in DCM cases characterized by elevated blood pressure [122].

Ca^{2+} channels antagonists. Verapamil exerts direct action on L-type calcium channels, thereby reducing intracellular calcium concentrations and alleviating myocardial damage in patients with DCM [174].

Management of coexisting cardiac diseases. Ideal treatment of hypertension and CAD is anticipated to mitigate the progression of the disease. Coronary intervention, when indicated for significant CAD, may alleviate symptoms and improve clinical outcomes. Management of heart failure is contingent upon factors like severity, and associated conditions such as hypertension and CAD [122].

1.3.4.3 Drugs in clinical trials for the treatment of DCM

AT-001 (Caficrestat) and Ninerafaxstat (IMB-1018972) are novel small-molecule chemical compounds. AT-001, which is an oral aldose reductase inhibitor targeting AKR1B1, is currently in phase 3 clinical development for DCM treatment. Clinical evaluation of Ninerafaxstat's effects is ongoing [141].

Trimetazidine, an atypical anti-anginal agent possessing antioxidant properties, modulates cardiac energy metabolism by favouring glucose oxidation over FFAs oxidation. Recent findings indicate that trimetazidine, acting as an inhibitor of Acetyl-coA Acyltransferase 2 (ACAA2), can ameliorate DCM by reducing fatty acid deposition, inhibiting Nox2/TRPC3-induced oxidative stress, attenuating fibrosis, decreasing apoptosis, and enhancing autophagy. The effects of trimetazidine on LV function and inflammatory markers in T2DM patients were assessed in a phase 2 clinical trial [122, 175-177].

Alpha-lipoic acid, possessing antioxidant properties, has demonstrated potential in mitigating cardiac remodelling in diabetic myocardium. While experimental models have illustrated the favourable impact of alpha-lipoic acid on cardiac redox homeostasis and suppression of cardiac fibrosis, human data confirming these effects are currently unavailable [178, 179].

Phosphodiesterase 5 Inhibitors (PDE5Is). Several trials have been conducted to evaluate the potential effects of phosphodiesterase type 5 inhibitors (PDE5i) on cardiac and renal function:

- Sildenafil, a selective PDE5i, has recently demonstrated improvement in myocardial remodelling, cardiac function, and certain circulatory markers of cardiac inflammation in patients with DCM [180].
- Tadalafil has shown promise in ameliorating cardio-renal complications associated with T2DM through continuous PDE5i therapy. This therapeutic protocol may mitigate diabetic microvascular complications, including albuminuria, erectile dysfunction, and myocardial remodelling in men, while also potentially offering protective effects for women at risk of diabetic nephropathy [181].

Spironolactone exhibits antifibrotic effects on the hearts of patients with T2DM, as evidenced by non-invasive cardiac imaging in a clinical trial [141].

Perhexiline, an inhibitor of carnitine 0-palmitoyltransferase 1, enhances maximal oxygen consumption (VO2 max), left ventricular ejection fraction, resting and peak stress myocardial function, and skeletal muscle energetics. Perhexiline also ameliorates metabolic impairment in DM prior to HF onset. However, despite these benefits, the utilization of perhexiline is diminishing due to reports of side effect such as hepatotoxicity and peripheral neuropathy [141, 182, 183].

1.3.4.4 New potential intervention strategies on heart protection in DCM

Oxidative stress plays a significant role in the pathogenesis of diabetic cardiomyopathy. Various studies have explored diverse strategies aimed at reducing the accumulation of reactive oxygen species (ROS). Initiating antioxidant supplementation early after the diagnosis of

diabetes mellitus warrants serious consideration as an adjunctive therapeutic approach to be integrated into the treatment regimen, with the aim of mitigating the risk of future CV complications [119, 140].

Sulforaphane, is an activator of the transcription factor Nrf2, that modulates the expression of several antioxidant proteins, has been shown to reduce ROS production in arterioles of diabetic mice. It also attenuates cardiac remodelling and dysfunction [184, 185].

Coenzyme Q10 has demonstrated efficacy in improving cardiac function in HF patients with DM. It mitigates oxidative stress and pathological myocardial remodelling. Coenzyme Q10 therapy reduces cardiac inflammation, fibrosis, and hypertrophy in mouse models of both T1DM andT2DM [123, 186].

Inflammatory pathways represent potential targets for the development of novel therapies aimed at mitigating the progression of DCM. For instance, the T cell-specific deletion of sphingosine 1-phosphate receptor 1 (S1PR1), along with the administration of the S1PR1 antagonist FTY720, has been demonstrated to provide protection against cardiac fibrosis in a STZ-induced diabetic model. [187].

NLRP3 gene silencing therapy has emerged as a promising approach for addressing DCM, as the NLRP3 inflammasome is implicated in metabolic disorders and cell death, pivotal factors in DCM pathogenesis. NLRP3 gene silencing therapy has demonstrated efficacy in ameliorating pyroptosis, fibrosis, cardiac inflammation, and cardiac function. Various drugs have been identified for their ability to interfere with the NLRP3 pathway in DCM. For instance, rosuvastatin, an anti-hyperlipidaemic agent, inhibits NLRP3 inflammasome activation via the MAPK pathway in DCM. Dapagliflozin, a SGLT2i inhibitor, suppresses NLRP3 inflammasome expression by enhancing AMPK phosphorylation, thereby alleviating DCM in mice. Additionally, metformin by activating the AMPK/autophagy pathway exerts cardioprotective and anti-inflammatory effects, consequently inhibiting the NLRP3 inflammasome in DCM [145, 188, 189].

miRNA, circRNA, and stem cell transplantation (SCT) represent novel cardioprotective interventions in diabetic DCM. Modulation of miRNAs can serve as a response to various pathological conditions, such as hyperglycaemia, hyperinsulinemia, oxidative stress, and inflammation. Dysregulation of miRNA function constitutes a significant pathogenic mechanism in DM and DCM. Therefore, restoring normal miRNA function holds promise as a potential therapeutic target. Certain miRNA targets have shown efficacy in treating structural heart disease in murine models. Recent scientific data supports the potential utility of exosomes and circulating miRNAs as biomarkers for detecting DCM, offering new way to diagnose and

prevent DM and DCM [115, 119, 190]. Likewise, transplantation of bone marrow-derived endothelial progenitor cells has demonstrated efficacy in ameliorating DCM in rodent models. Ongoing research endeavours aim to translate the data observed in these experimental models into clinical practice in the future [191].

Gene therapy holds promise as a future therapeutic approach, offering the ability to modulate the expression of specific cardiac genes *in vivo*. For instance, gene delivery of nerve growth factor has been shown to preserve LV systolic and diastolic function, microvessel density, and myocardial perfusion [145, 192].

Sirtuins, particularly SIRT1, present a promising therapeutic target for reducing and preventing DCM, owing to their protective effects against CVD by combating sustained oxidative stress [193]. Tetrahydrocurcumin administration has been shown to upregulate SIRT1, thereby attenuating pathological downstream transformations, such as the deacetylation of SOD2 [194]. Additionally, activation of SIRT3, in conjunction with melatonin administration, has demonstrated a similar protective effect in reducing hyperglycaemia-induced oxidative stress [195].

Traditional Chinese medicine and some natural products. Certain traditional Chinese medicines and natural products, including ginsenoside, resveratrol, berberine, curcumin, fenugreek, epigallocatechin gallate, flavonoids, *Ginkgo biloba* extract, and *Astragalus* polysaccharide, have been scientifically demonstrated to possess anti-diabetic, hypocholesterolaemic, antioxidant, anti-inflammatory, and antiapoptotic properties. These compounds exert their effects by modulating myocardial function in DCM models [196-199].

Despite increasing interest in the pathophysiology of DCM, specific guidelines for patient diagnosis or treatment strategy implementation in clinical practice are lacking. Currently, treatment approaches rely on managing underlying DM and addressing risk factors associated with CVD progression [119].

1.4 The involvement of select traditional herbal extracts such as fenugreek and horsetail in the prevention and treatment of obesity, T2DM and diabetic cardiomyopathy

1.4.1 Fenugreek (*Trigonella foenum-graecum* L.)

1.4.1.1 General presentation

Trigonella foenum-graecum L. commonly known as fenugreek, is a self-pollinating plant that typically grows once a year. Species of Trigonella are widely distributed across various regions worldwide, including Asia (India and China), parts of Europe, Africa, Australia, and North and South America. It belongs to the subfamily Papilionaceae, family Leguminosae (Fabaceae). The genus name, Trigonella, originates from the Greek word meaning “three angled”, while the term “fenugreek”, derived from “foenum-graecum”, translates to “Greek hay” [200].

Trigonella foenum-graecum L. is also included in pharmacopoeias. It possesses a complex phytochemical composition with diverse therapeutic activities. Fenugreek seeds contain a diverse array of constituents, including carbohydrates such as galactomannan, proteins, and amino acids like 4-OH isoleucine. They also comprise lipids such as phospholipids, glycolipids, oleic acid, and linoleic acid, along with alkaloids, flavonoids, fibres, saponins, and steroidal saponins. Additionally, fenugreek seeds contain vitamins A, B1, B2, and C, as well as choline, nicotinic acid, and niacin. Minerals and nitrogen compounds are also present, with constituents falling into both non-volatile and volatile categories [1, 200, 201].

Scientific studies have documented various medicinal uses of fenugreek seeds, including their potential as remedies for diabetes and hypercholesterolemia, as well as their hepatoprotective effects and their role in protecting against breast and colon cancer. Fenugreek seeds are also known for their anti-inflammatory properties and are used as detoxifiers, in managing abdominal cramps during diarrhoea, treating gastric ulcers, and alleviating symptoms of fever and respiratory diseases such as sinusitis. Additionally, fenugreek seeds are employed as emollients for the skin and as galactagogues to promote lactation. These protective effects are attributed to the presence of non-nutritive secondary metabolites, also known as phytochemicals [200]. The therapeutic effects of fenugreek seeds are detailed in Table IV, categorized according to their nutritional components.

Table IV. *Nutritional/health impacts of TFG components [202]*

Nutritional components	**Content**	**Health consequences.**
4-hydroxisoleucine (amino acid)	0.09% [203]	– stimulates insulin activity – [202] – [204]
Fibre (soluble dietary fibre, galactomannans, mucilage, tannins, pectin and hemicellulose, non-starch polysaccharides)	50–65 g/100g [201]	– binds food toxins – inhibit bile salt absorption in the colon – facilitate LDL level reduction in the plasma – protects the colon mucus membrane – promotes insulin secretion – water retention in the intestine – lower the blood glucose absorption – [201] – [202]
Phenolic acids	101.4 mg/100g [205]	– antioxidant properties – [201, 205]
Flavonoids	more than 100 mg [201]	– antioxidant properties – [201]
Micronutrients (Vitamins and minerals)	784 mg/100g [201]	– Regulatory functions – [202]
Diosgenin	0.1–0.9% [203]	– anti-inflammatory [206] – anti-carcinogenic [206] – hepatoprotective [202] – hipolipidaemic [203]

Treatment with fenugreek seeds offers multiple benefits for patients with DM. Research conducted over the last twenty years has demonstrated the positive effects of fenugreek seeds on blood glucose levels and glucose tolerance in individuals with DM. TFG has been shown to significantly impact the metabolism of lipids and glucose, exert insulin-sensitizing effects, possess antioxidant properties, and contribute to maintaining energy balance. These benefits in DM, IR, and obesity are attributed to the components of fenugreek seeds such as galactomannan, 4-OH isoleucine, and diosgenin [207].

The antidiabetic activity of TFG, both in vivo and in vitro, exhibits dose-dependent characteristics, and the formulation of pharmaceutical products containing TFG also plays a significant role in determining its therapeutic benefits [224].

The role of fenugreek leaves and seeds in combating hyperlipidemia, liver dysfunction, and IR is illustrated in Figure 10 [202].

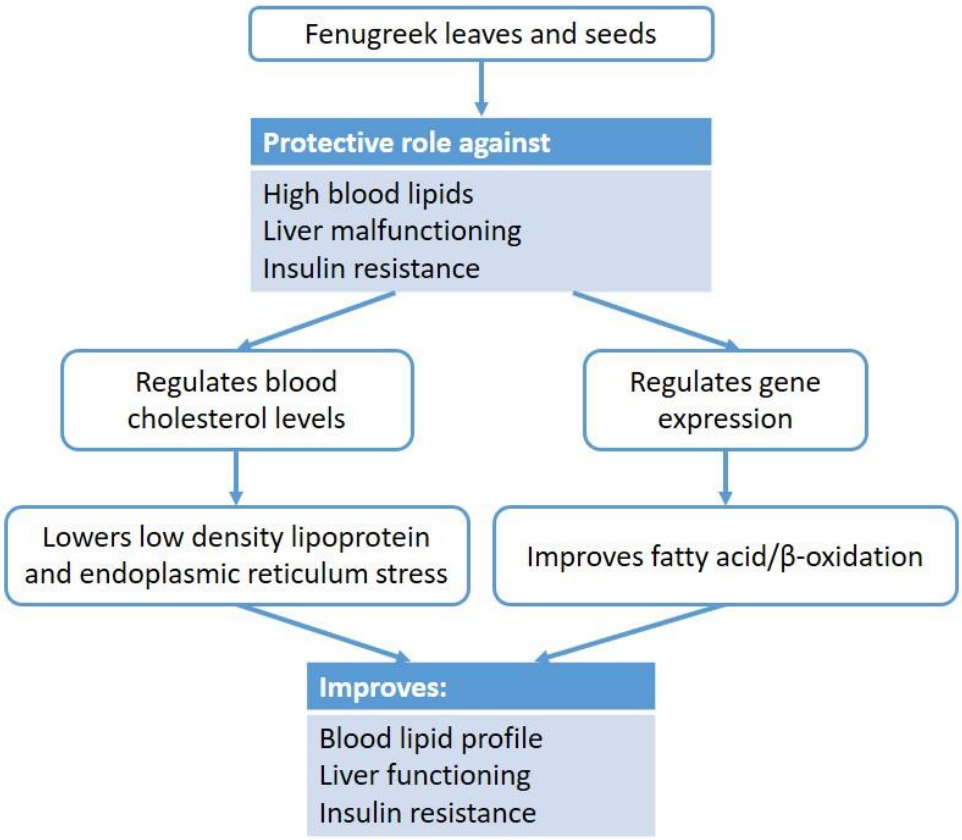

Figure 10. *Role of TFG leaves and seeds against hyperlipidaemia, liver malfunctioning, and IR [202]*

While fenugreek is generally considered safe, certain adverse effects have been reported. Transient diarrhoea, dizziness, and flatulence are among the side effects associated with TFG consumption. Additionally, TFG supplementation may lead to hypoglycaemia, necessitating monitoring of blood glucose levels. Consumption of TFG extracts has been linked to decreased production of T3 (triiodothyronine). Furthermore, fenugreek preparations containing coumarin derivatives may increase the risk of bleeding by prolonging prothrombin time and the international normalized ratio (INR). TFG should be avoided during pregnancy due to its potential to stimulate uterine contractions, as observed in animal studies. Moreover, concurrent use of fenugreek with certain medications may potentiate hypokalaemic, hypoglycaemic, and estrogenic effects [202].

1.4.1.2 Diosgenin and its therapeutic activities in obesity, insulin resistance and T2DM

Diosgenin, a biologically active steroid sapogenin found in TFG, exhibits beneficial therapeutic effects against various pathologies including diabetes, hyperlipidemia, cancer, CVD, oxidative stress, and inflammation. Orally administered diosgenin is generally well tolerated at doses of up to ~500 mg/kg in alcoholic extracts [208, 209].

Recent studies using animal models of diabetes support the role of diosgenin as an antidiabetic

agent, demonstrating its ability to reduce blood glucose and restore insulin sensitivity [208, 210]. Diosgenin exerts its effects on various organs including the pancreas, liver, skeletal muscle, and adipose tissue, leading to the restoration of insulin sensitivity, glucose homeostasis, and normalization of blood lipids in diabetic animals. The effects of diosgenin and other bioactive compounds from TFG on these organs are illustrated in Figure 11 [208].

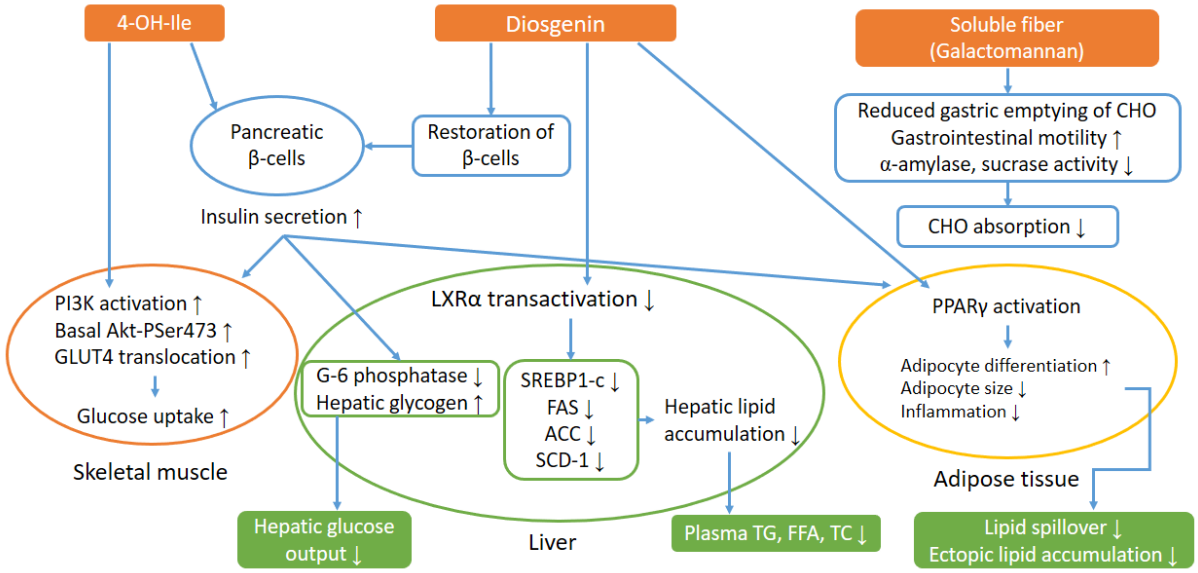

***Figure 11**. Bioactive compounds of TFG and their mechanism of action [208]*

Diosgenin, through mechanisms such as restoration of pancreatic β-cell function, attenuation of pancreatic ER and oxidative stress, and activation of PPAR-γ in adipose tissue, has been demonstrated to enhance insulin secretion and maintain normal blood glucose levels [208, 210]. Due to its anti-inflammatory properties, diosgenin has exhibited protective effects on the kidneys of diabetic rats, mitigating renal complications associated with T2DM [211].

In a diabetic rat model, diosgenin induces alterations in the lipid profile of various tissues, including plasma, liver, heart, and brain. These changes may be associated with its hypoglycaemic effects [209]. Emerging evidence suggests that apart from its role in insulin regulation, TFG, specifically diosgenin, may also modulate the synthesis and function of other metabolic hormones such as IGF-1, GH, T3, and thyroxine (T4). These hormones are crucial in regulating glucose metabolism and the pathogenesis of T2DM [209].

In obesity, adipocyte hypertrophy and chronic inflammation in adipose tissues contribute to IR and the development of T2DM. TFG, containing diosgenin, could potentially mitigate glucose metabolic disorders associated with obesity by promoting adipocyte differentiation and suppressing inflammation in adipose tissues [209, 212].

1.4.2 Horsetail (*Equisetum arvense* L.)

1.4.2.1 General presentation

Equisetum arvense L, commonly known as field horsetail, is a perennial fern belonging to the Equisetaceae family [213]. It is characterized by black rhizomes and two distinct types of stems, namely spring and summer stems. The green, photosynthetic sterile stems are heavily branched and are produced in late spring, persisting until late autumn. Notably, the sterile stem is the medicinal component of the plant, referred to as Equiseti herba, as mentioned in the European Pharmacopeia (Ph. Eur. 8) [213]. Among the species within the Equisetum genus, only *Equisetum arvense* L., or "Equiseti herba", is listed in the German commission E Monograph for phytotherapy and herbal substances, as well as in the European Pharmacopoeia [214]. Field horsetail thrives spontaneously in light sandy soils and is distributed across various regions including Europe, Africa, South America, Southern Asia, Turkey, and Iran [215].

It contains various chemical compounds such as glucosides, flavonoids, saponosides, phytosterols, sterols, , triterpenoids, silicic acid, linoleic acid, oleic acid, stearic acid, and traces of alkaloids, calcium carbonate, potassium sulphate, potassium chloride, manganese chloride, iron, manganese and calcium phosphate, vitamin C, proteins and amino acids, volatile oils [214, 216].

Pharmacological studies have revealed that *Equisetum arvense* L. possesses a broad spectrum of therapeutic effects, including antioxidant, anti-tumour, antimicrobial, smooth muscle relaxant, anticonvulsant, anxiolytic, sedative, dermatological, immunological, analgesic, anti-inflammatory, antidiabetic, diuretic, anti-platelet, promotion of osteoblastic response, anti-leishmanial activities, among others [217]. Traditionally, *Equisetum arvense* L. has been utilized for tuberculosis, as a remedy for urinary tract inflammation and infections, as a haemostatic agent for profuse menstruation, nasal, pulmonary, and gastric haemorrhages, for conditions such as brittle fingernails and hair loss, as well as for rheumatic diseases, gout, poorly healing wounds and ulcers, swelling and fractures, and for the treatment of frostbite [217].

Equisetum arvense L. exhibits antioxidant effects attributed to its phenolic compounds, which confer potent protection against free radicals, lipid peroxidation, and oxidative agents. This property has been supported by research findings [218]. Furthermore, the presence of high concentrations of flavonoids, phenolic compounds, and mineral salts suggests a mild diuretic action of *Equisetum arvense* L. Additionally, its abundance of silicon salts suggests potential remineralisation properties [219].

The plant can be utilized for internal therapy through preparations such as infusions, alcoholic extracts, capsules, as well as for external use in the form of ointments.

In acute toxicity studies, extracts from the plant exhibited no adverse effects, with no mortalities observed in rats even at doses up to 5000 mg/kg body weight. Similarly, in subacute toxicity studies, rats fed diets containing 0.3%, 1%, and 3% *Equisetum arvense* L. powder showed no significant changes in body weight, cumulative body weight gains, or biochemical and haematological parameters. Furthermore, *Equisetum arvense* L. demonstrated no potential for chromosomal aberrations or mutagenicity in vivo. Regarding the safety of consuming horsetail during pregnancy or while breastfeeding insufficient information exists [217].

In experimental studies with rats, acute hepatotoxicity was assessed following administration of varying doses of *Equisetum arvense* L. extract over a 14-day period. Long-term oral consumption of the plant is deemed potentially unsafe, as it contains thiaminase, an enzyme that breaks down thiamine. This may result in thiamine deficiency, highlighting a potential health risk associated with prolonged ingestion [217].

The onset of poisoning from *Equisetum arvense* L. occurs gradually and is characterized by manifestations such as unkempt physical appearance, diarrhoea, and mild incoordination. Treatment strategies should focus on eliminating the source of poisoning. In cases of poisoning, initial administration of thiamine (vitamin B1) intravenously followed by intramuscular injections for several days may be warranted [217].

Horsetail is contraindicated in individuals with alcoholism, who are often thiamine deficient, as its consumption may exacerbate thiamine deficiency. Additionally, horsetail may lead to potassium depletion, necessitating caution when used in patients at risk for potassium deficiency. Patients with oedema stemming from impaired heart and kidney function should also avoid horsetail. Products containing horsetail should be used cautiously in cases of significant skin lesions, acute lesions of unknown origin, severe febrile or infectious diseases, cardiac insufficiency, and hypertension[220].

1.4.2.2 The antidiabetic and cardioprotective effects of horsetail extract

Due to its high concentration of flavonoids, *Equisetum arvense* L. exhibits potent radical scavenging activity against superoxide anion and hydroxyl radicals. This antioxidant capacity plays a crucial role in protecting the human body from damage caused by ROS, lipid peroxidation, and oxidative agents, common in conditions such as diabetes, atherosclerosis, and ischemic heart disease [218, 220, 221].

In STZ-induced diabetic rats, treatment with methanolic extract of *Equisetum arvense* L. at various doses for 5 weeks, significantly reduced blood glucose levels and promoted

regeneration of the necrotized pancreas [217, 222-224]. Recent studies on *Equisetum arvense* L. extract have elucidated the connection between its flavonoid compounds and SIRT1 in mediating antidiabetic effects and cardioprotection, owing to its antioxidant capacity.

1.4.2.3 Effects of horsetail extract on SIRT1 activity

Numerous studies have illustrated that *Equisetum arvense* L. extract contains flavonoids, which have the potential to enhance SIRT1 expression through their anti-oxidative effects. SIRT1, a regulatory protein, governs various metabolic pathways, cell survival mechanisms, cellular senescence, and inflammatory processes. It contributes to the pathogenesis of chronic d such as diabetes, pulmonary, neurodegenerative, and CVD. This regulatory role is achieved through the deacetylation of lysine groups on histone and non-histone proteins, including notable transcription factors such as FOXO, p53, MyoD, and PGC-1α [225, 226].

Flavonoids are recognized as promising agents for preventing and treating oxidative diseases associated with stress. Among them, quercetin stands out as the most extensively studied flavonoid due to its rapid and efficient absorption, coupled with its antioxidant, anti-inflammatory, antidiabetic, cardioprotective, and antiviral properties. *Equisetum arvense* L. extract is notably rich in quercetin, which has been shown to ameliorate IR and enhance glucose metabolism via the activation of SIRT1. Moreover, studies have demonstrated that quercetin exhibits protective effects against oxidative damage in STZ-induced diabetic rats by upregulating SIRT1 expression and downregulating the levels of NF-κB, a known substrate of SIRT1 [225].

ROS are pivotal contributors to endothelial cells (ECs) impairment and subsequent endothelial dysfunction, which underlie the development of CVD. Quercetin exerts a cardioprotective effect against endothelial dysfunction by activating SIRT1, thereby inhibiting ECs damage induced by oxidized LDL [226].

Numerous studies conducted on HFD-induced obese mouse models have elucidated the effects of quercetin on adipocytes and adipose tissue in relation to obesity. These studies indicate that quercetin can suppress the inflammatory response of macrophages by activating AMPK phosphorylation and upregulating the expression of SIRT1 [227].

Studies conducted on HFD-induced obese mice models have investigated the effects of quercetin on adipocytes and adipose tissue in relation to obesity. These studies have revealed that quercetin can mitigate the inflammatory response of macrophages by promoting AMPK phosphorylation and enhancing the expression of SIRT1 [227].

1.5 The role of SIRT1 in T2DM and DCM

Sirtuins comprise a set of evolutionarily conserved enzymes categorized as NAD+-dependent histone and protein deacetylases (SIRT1, SIRT3, and SIRT5) and/or ADP-ribosyl transferases (SIRT4 and SIRT6), exerting crucial roles in various biological processes. They derive their name from their resemblance to the Saccharomyces cerevisiae gene silent information regulation-2 (Sir2) and are classified as class III histone deacetylases (HDACs) [228-230]. Seven mammalian homologs of sirtuin (SIRT1-7) have been identified, distributed across distinct subcellular compartments, including the nucleus (SIRT1, 2, 6, and 7), cytoplasm (SIRT1 and 2), and mitochondria (SIRT3, 4, and 5) (Figure 12.) [231-233].

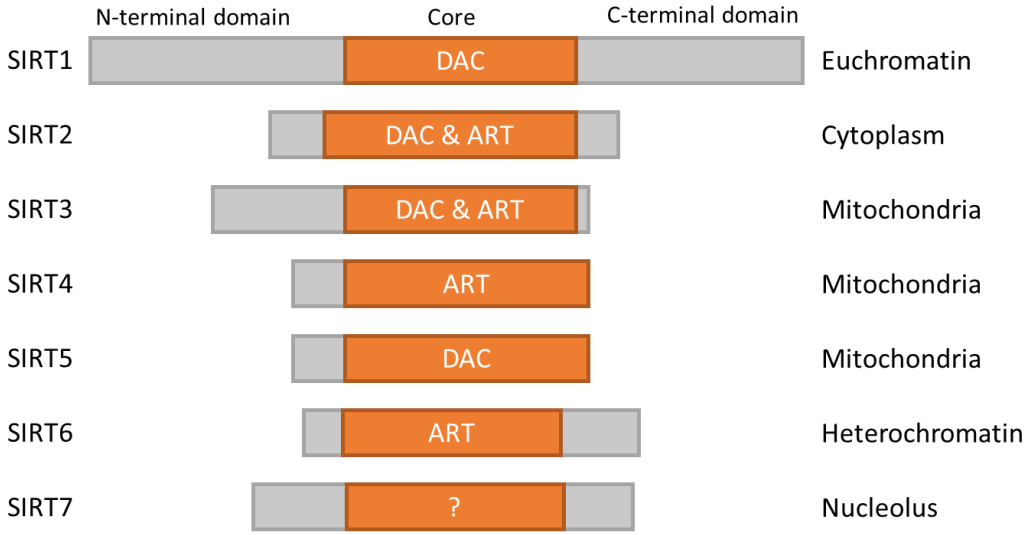

Figure 12. *Intracellular localization and enzymatic activity of sirtuins. DAC: Deacetylase; ART: ADP-ribosyltransferase. [234]*

Sirtuins are widely expressed across various tissues, including the brain, spinal cord, dorsal root ganglia, hypothalamus, pancreatic ß-cells, liver, skeletal muscles, and adipocytes. Sirtuins perform crucial roles in various biological processes such as cellular stress response, DNA repair, genome stability, cell cycle regulation, cell survival, and maintenance of cellular homeostasis, particularly within metabolic pathways, oxidative stress, inflammation, and aging. (Figure 13.) [233, 235-237].

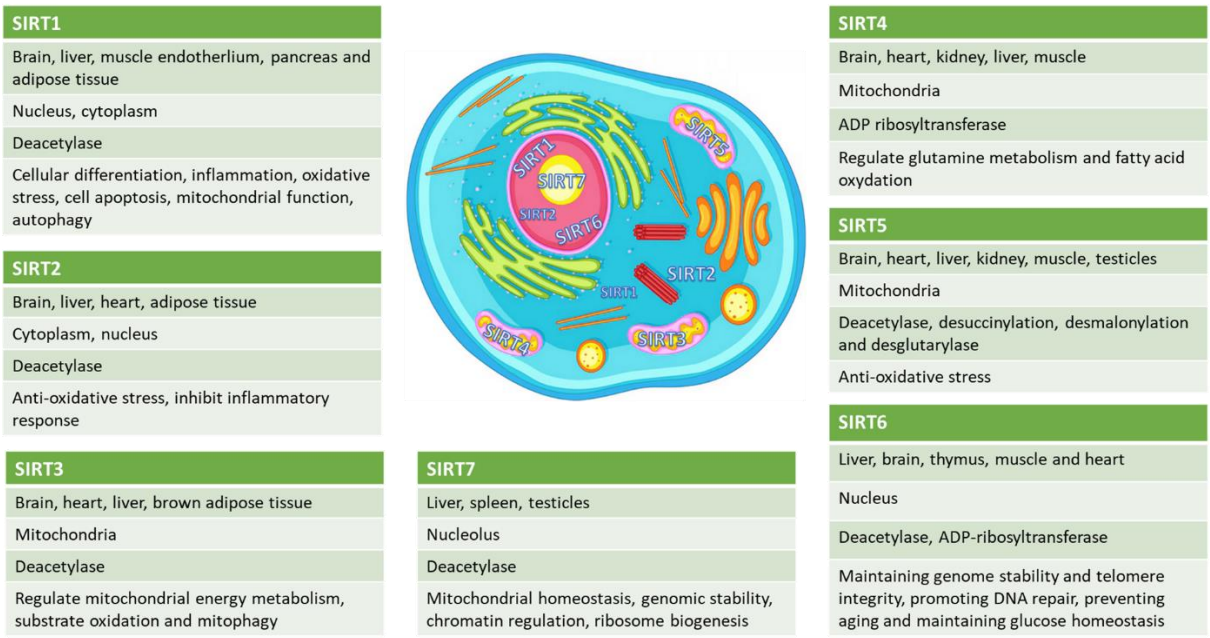

Figure 13. *Intracellular localization and tissue expression of sirtuins.*

Among the Sirtuin family, SIRT1 stands out as the most extensively studied member. Its therapeutic effects encompass various domains: enhancing insulin sensitivity, improving glycaemic control, mimicking calorie restriction, regulating lipid homeostasis across the liver, adipose tissues, and skeletal muscles to mitigate hyperlipidemia, exerting anti-inflammatory properties, shielding against CV events and endothelial dysfunction, promoting autophagy and apoptosis, combating cancer, and exerting anti-aging effects. (Figure 14.) [238].

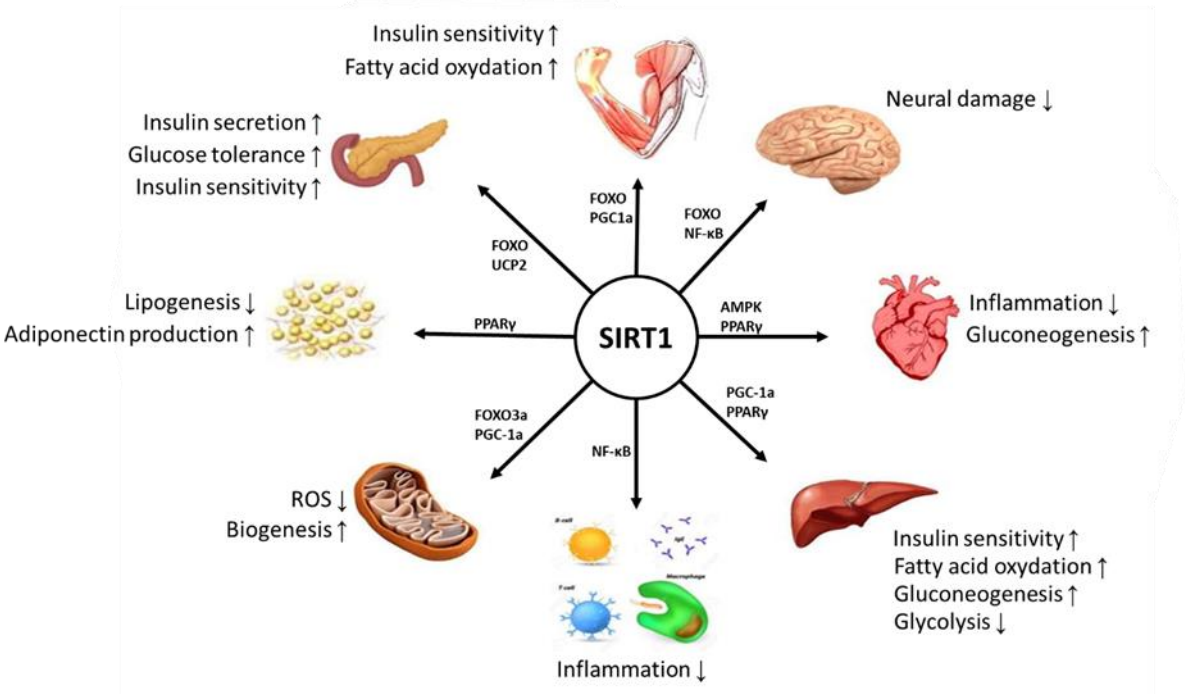

Figure 14. *Effects of SIRT1 on organs and tissues at the level of some transcription factors.*

Over the past two decades, there has been significant research interest in exploring the therapeutic potential of sirtuins, particularly SIRT-1, in the prevention of T2DM. Uncoupling Protein 2 (UCP2) modulates the efficiency of insulin secretion. This protein is situated in the inner membrane of mitochondria, where it uncouples the electrochemical proton gradient, reduces ATP production, thereby preventing insulin secretion [239]. By directly binding to UCP2 promoter SIRT1 reduces the expression of the gene encoding UCP2. Local overexpression of SIRT1 in pancreatic β-cells results in a high level of insulin secretion [240], however, the role of SIRT1 in increasing insulin production is not yet fully understood. Various research groups support different theories regarding the involvement of SIRT1 in carbohydrate metabolism regulation. Moynihan et al. demonstrated that in SIRT1 Knock-Out (SIRT1-KO) mice and in pancreatic β-cell lines where the SIRT1 gene was knocked down by RNA interference, insulin secretion was reduced. This can be attributed to the inhibition of UCP2 by SIRT1 in pancreatic islet β-cells [241]. In SIRT1-KO mice, the quantity of UCP2 protein was increased, while serum insulin levels were low [240]. Conversely, UCP2-KO mice showed increased insulin secretion and ATP production [242]. Increased expression of UCP2 inhibits glucose-stimulated insulin secretion. Thus, SIRT1 acts more as a positive regulator than a suppressor of insulin secretion. Through activation of FOXO1, SIRT1 protects β-cells from damage-induced apoptosis. In response to oxidative stress or toxins, FOXO1 regulates the expression of multiple genes, thereby contributing to the preservation of insulin secretion and promotion of cell survival. However, inhibition of SIRT1 is associated with β-cell apoptosis [243, 244].

Cellular insulin sensitivity is an important aspect of carbohydrate metabolism. Protein tyrosine phosphatase 1B (PTP1B) holds a pivotal role in glucose metabolism, proved by PTP1B-deficient mice research which demonstrate heightened insulin sensitivity, enhanced glucose metabolism, and resistance against diet-induced obesity [245]. PTP1B, responsible for phosphorylating tyrosine residues on the insulin receptor, can undergo inhibition via deacetylation. Resveratrol, acting as a sirtuin activator, also possesses the capability to inhibit PTP1B. Consequently, SIRT1 can improve insulin sensitivity by diminishing PTP1B activity [246]. Additionally, SIRT1 expression in muscle cells can modulate insulin sensitivity through suppression of PTP1B protein transcription (a negative regulator of insulin signalling). Meanwhile, within adipose tissue, SIRT1 can regulate insulin-triggered glucose uptake by influencing GLUT4 translocation [247].

Overall, SIRT1 may contribute to the regulation of glucose homeostasis through various mechanisms. These include: modulating insulin secretion and protecting pancreatic β-cells,

enhancing insulin sensitivity by influencing post-insulin receptor signalling, reducing inflammation and lipid accumulation, regulating adiponectin secretion, modulating FA oxidation and mitochondrial biogenesis, and controlling hepatic glucose production and circadian rhythms (Figure 14.) [248, 249].

DCM stands as one of the most serious complications of diabetes, yet its precise pathomechanism remains unclear. Hyperinsulinemia and hyperglycaemia are pivotal in the mechanisms underlying DCM. These factors contribute to elevated levels of AGEs, triggering a cascade of degenerative processes including oxidative stress, heightened inflammation, fibrosis, hypertrophy, and apoptosis. Consequently, these events culminate in myocardial damage and the development of cardiomyopathy [193].

Signal transduction pathways such as ERK1/2/Homer1a/SIRT1, AMPK/SIRT1, SERCA2a/UPR/SIRT1, FOXO3a/SIRT1, NF-κB/SIRT1, and eNOS/SIRT1 are pivotal in the pathophysiology of DCM. SIRT1 modulates transcriptional factors including p300, NF-κB, P38MAPK, Histone 3, MMP-9, FOXO3a, and p53, while concurrently enhancing SERCA2a, ERK1/2/Homer1, eNOS, PGC-1α, and AMPK. Through these mechanisms, SIRT1 mitigates cardiac dysfunction and ameliorates DCM [193]. Investigations highlight the significant role of SIRT1 in the genesis and progression of DCM, presumably attributable to its antidiabetic, antioxidant, anti-inflammatory, antiproliferative, and anti-apoptotic properties [250].

SIRT1, highly responsive to cellular redox states, offer cardiac protection and preserves vascular function by mitigating the effects of ROS by deacetylation of numerous cellular targets. Advancements in understanding SIRT1 and SIRT6 signalling in CVD protection reveal their dependence on the cellular redox state. This underscores the potential of antioxidant compounds in CVD protection by acting on the SIRT1/FOXOs, SIRT1/NF-κB axis, SIRT1/p66Shc, and SIRT6/NF-κB axis [228].

Calorie restriction (CR) has emerged as a promising strategy for delaying the onset of various age-related diseases, including T2DM and DCM [249]. SIRT1, known to be upregulated by calorie restriction (CR), is closely associated with the anti-aging effects observed during CR [248]. Recent research conducted on animal models indicates that caloric restriction (CR) exerts a cardioprotective effect by promoting its catabolic activity and activating the expression of adaptive genes. This protective effect encompasses antioxidant defence mechanisms and is facilitated by the action of SIRT1 and the transcriptional coactivator PGC-1α [251].

ER stress-induced apoptosis has been observed in the diabetic heart. SIRT1 may alleviate this process by attenuating ER stress-induced cardiomyocyte apoptosis through pathways mediated by PERK/eIF2α, ATF6/CHOP, and IRE1α/JNK [252].

2 Aims of the Thesis

The incidence and prevalence of obesity and T2DM continue to rise, despite the introduction of novel therapeutic agents in recent years. Complications of diabetes, including microvascular and macrovascular complications, contribute to the onset of DCM. Despite the fact that the treatment of DCM is very complex, current therapeutic strategies lack specificity and adequate efficacy. In our days, research has shifted towards the exploration of traditional medicinal approaches for identifying new alternative compounds with reduced adverse effects and interactions.

The **general aim of the thesis** was to investigate the potential of select traditional medicinal plants in the pathophysiology of obesity, T2DM, and DCM. Furthermore, we aim to explore their pharmacological effects in preventing and treating these conditions, as well as elucidate the underlying signalling pathways, involved in the pathogenesis of diabetes mellitus and diabetic cardiomyopathy.

In our first investigation, we examined the effects of chronic oral administration of fenugreek seeds and diosgenin, one of its saponins, on diet-induced obese rats. Over a six-week treatment period, we monitored daily changes in body weight, food and water intake. Insulin sensitivity was assessed via an insulin tolerance test at the study endpoint. We aimed to evaluate the antidiabetic and anti-adipose effects of these treatments.

In our second investigation, we examined the effects of chronic horsetail extract administration in a STZ-induced diabetic rat model. Our investigation into plant extracts' effects on insulin sensitivity and the prevention and treatment of diabetes complications, including DCM, led us to include Horsetail extract (*Equisetum arvense* L.) in our study. With an aging population and the potential anti-aging and health-enhancing benefits associated to sirtuins, numerous studies are concentrating on identifying sirtuin activators, predominantly sourced from plant-derived compounds. Consequently, our objective is to investigate the influence of field horsetail, an area of inquiry that has not been explored previously, on SIRT1. By investigating the potential modulation of insulin sensitivity in STZ-induced diabetic rats through SIRT1 activation by *Equisetum arvense* L. extract, we aim to uncover new signalling pathways for the development of more effective antidiabetic and cardioprotective agents.

In conclusion, these researches may pave the way for the development of more effective antidiabetic or anti-obesity drugs.

3 Experimental design of the Thesis

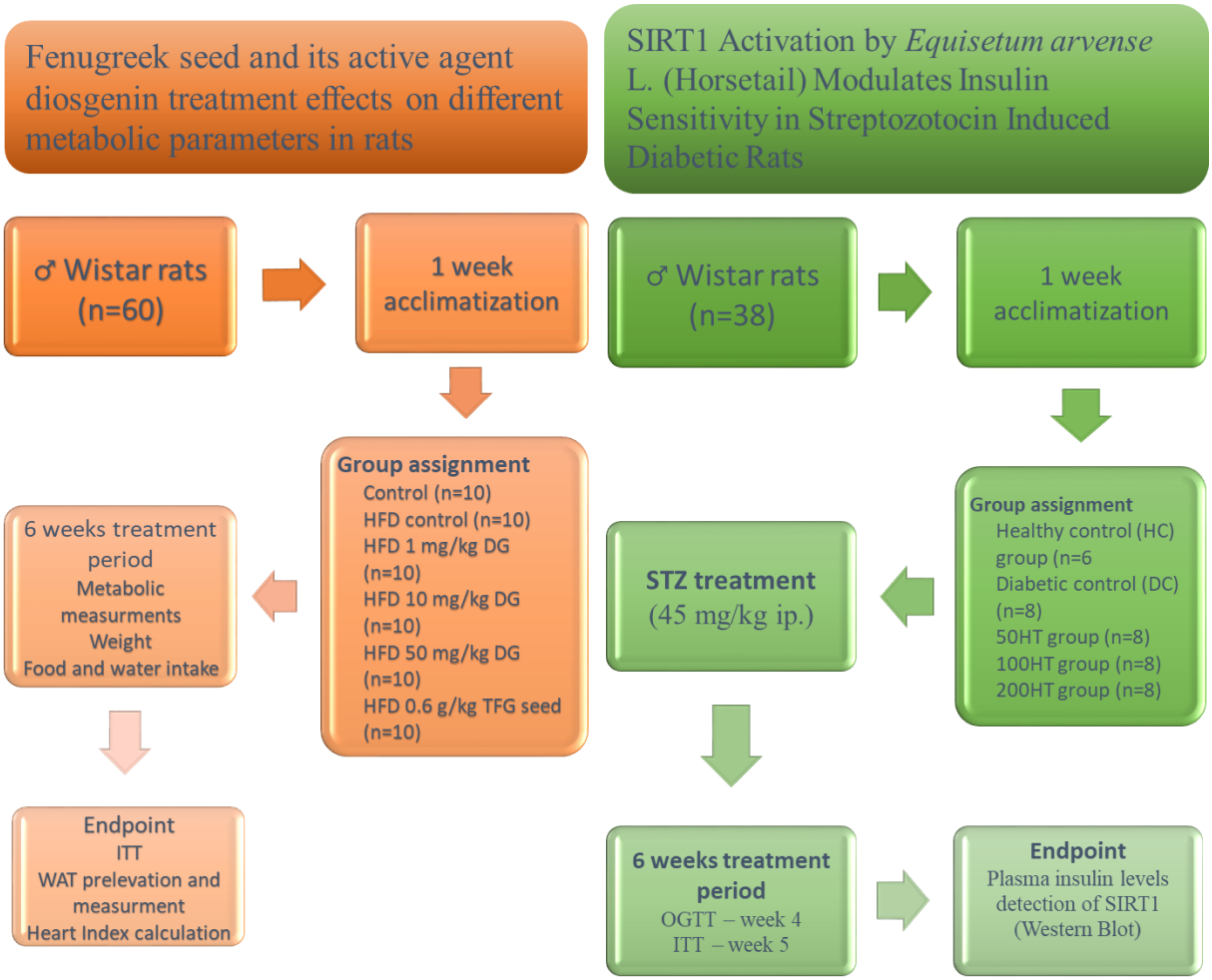

Figure 15. *Experimental design of the Thesis*

4 Materials and Methods

4.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats

4.1.1 Ethics

The study adhered to the ethical principles outlined in Declaration of Ethics in Decommissioning 08/2007 DE MÁB and 16/2007 DE MÁB. Additionally, it complied with international guidelines (published in 1996 by the National Academy Press, located at 2101 Constitution Ave. NW, Washington DC 20055, USA.) for the treatment of experimental animals, as recommended by the European Union and the United States.

4.1.2 Animals

To explore the effects of chronic oral administration of TFG seeds and diosgenin (DG), we utilized a diet-induced obesity model comprising 60 male Wistar rats. The animals were housed in an animal room maintained at a temperature of 22 - 24°C and relative humidity of 50 - 70%. The lighting schedule followed a 12-hour light-dark cycle. Following a one-week acclimatization period, the rats were randomly divided into six groups. Three rats from each group were individually housed in metabolic cages (model 3701M081, Tecniplast, Italy), while the remaining animals were group-housed in standard rat cages with 3 - 4 rats per cage. The control group received ad libitum access to standard laboratory chow (S8106-S011 SM R/M-Z+H, ssniff Spezialdiäten GmbH, Germany) and tap water, while the other five groups were subjected to a diet-induced obesity regimen.

4.1.3 Research Protocol

After the acclimatization period the experimental animals were assign randomly in 6 groups as follows: 1 healthy control, 1 HFD control, and 4 groups treated with various doses of diosgenin (1mg/kg, 10 mg/kg and 50mg/kg) and appropriate dose of TFG seeds. During the 6 weeks of treatment, the animals housed in metabolic cages were monitored daily, regarding the body weight, food and water consumption, urine and stool production, while the animals housed in standard cages were monitored for body weight (twice a week), and daily for food and water consumption. At the endpoint of the experiment ITT were performed, followed by the scarification of animals and prelevation of samples for further investigations (Figure 16.).

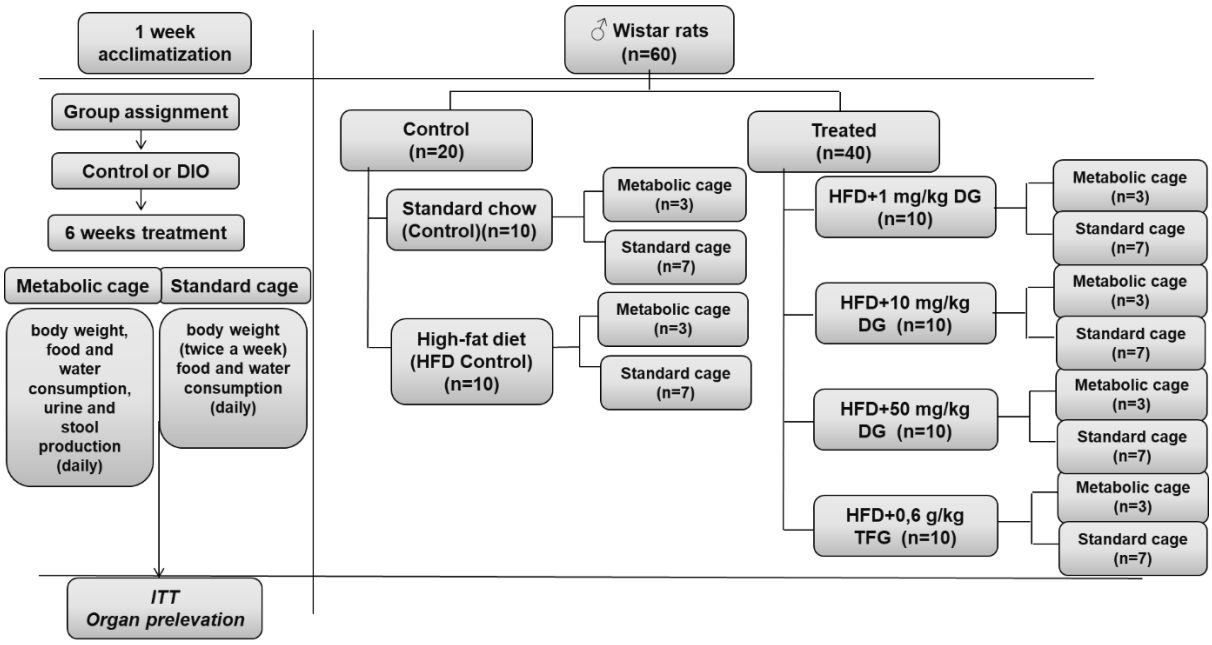

Figure 16. *Research Protocol*

4.1.4 Induction of Obesity

In the experiment involving TFG seeds and diosgenin, obesity was induced using a high-fat diet formulated for rodents, specifically RM AFE 45% FAT 20% CP 35% CHO (P) from Special Diets Services, UK, supplemented with a 5% sucrose solution. Different concentrations of diosgenin (1, 10, and 50 mg/kg) or TFG seeds (0.6 g/kg) were incorporated into the chow provided to the treated diet-induced obesity (DIO) rats (Table VIII.). The diosgenin was obtained from Sigma-Aldrich, Budapest, Hungary and the fenugreek seeds from Trigonella Med. Ltd., Mosonmagyaróvár, Hungary. The duration of the experiment spanned 6 weeks.

Table VIII. *Composition of prepared foodstuffs per kg of weight*

Groups	Diet				
Control group	Standard chow				
	DG/TFG (mg)	High-fat diet chow (g)	Sesame oil (mL)	Gelatine (g)	Water (mL)
HFD control group	0	854.7	14.25	6.55	124.5
HFD 1 mg/kg DG	15.7	854.7	14.25	6.55	124.5
HFD 10 mg/kg DG	156.7	854.7	14.25	6.55	124.5
HFD 50 mg/kg DG	783.5	854.7	14.25	6.55	124.5
HFD 0.6 g/kg TFG seed	18201.9	827.36	13.79	7.03	133.62

4.1.5 Metabolic measurements

For the animals housed in metabolic cages, measurements of body weight, food and water consumption, as well as urine and stool production, were conducted daily. During weekends, a

3-day average was calculated on Monday mornings. Rats housed in standard cages had their body weights measured twice a week, with daily monitoring of food and water consumption. The dosage of diosgenin (DG) or fenugreek seeds (TFG) incorporated into the chow was determined based on weekly data of body weight and food consumption. Daily calorie intake was calculated using the following formula: standard chow (3.2 kcal/g), high-fat diet (4.56 kcal/g), and 5% sucrose solution (0.2 kcal/mL). At the end of the experiment the abdominal (retroperitoneal, gonadal) white adipose tissue (WAT) was removed and measured.

4.1.6 **Determination of insulin sensitivity**

On week 6, an insulin tolerance test (ITT) was conducted. Prior to the experiment, the animals underwent a 3-hour fast, following which the basal blood glucose levels were determined via tail clipping. Blood glucose concentration was assessed using a glucometer (Accu-Chek, Roche Diagnostics, Budaörs, Hungary). Subsequently, insulin was administered intraperitoneally at a dosage of 0.5 U/kg. Blood glucose levels were then measured at 30, 60, 90, and 120 minutes post-insulin injection. Insulin tolerance was evaluated based on the area under the glucose curve.

4.1.7 **Statistics**

All data were subjected to analysis using one-way analysis of variance (ANOVA), followed by a modified t-test for repeated measures as per Bonferroni's method.

4.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats

4.2.1 Ethics

Samples of *Equisetum arvense* L. were sourced from unpolluted areas within the indigenous flora of Oradea and provided by Prof. Annamaria Pallag (Pharm.D) from the Department of Pharmacy, University of Oradea, Faculty of Medicine and Pharmacy, located at 1st December Square 10, Oradea, 410068, Romania.

Our study adhered to the guiding principles of the European Community and the University of Debrecen Ethics Committee for Animal Research regarding the care and use of experimental animals. The ethical code number assigned to our study was 29/2017/DEMÁB, with the ethical submission approved on 6 March 2018.

4.2.2 Animals

To assess the involvement of SIRT1 in the pathomechanism of diabetes and diabetic cardiomyopathy, as well as to explore the effect of *Equisetum arvense* L. on these pathological conditions male Wistar rats were employed. The rats, obtained from TOXI-COOPZRT., Budapest, Hungary, were 6-7 weeks old and weighed between 175-200g. They were housed in controlled conditions at 22–24°C with relative humidity maintained at 50%–70%, and subjected to a 12–12-hour light/dark cycle. The rats were provided with standard laboratory chow (S8106-S011SMR/M-Z+H; ssniff SpezialdiätenGmbH, Soest, Germany) and tap water ad libitum. Following a one-week acclimatization period, the animals were randomly assigned to five groups: one healthy control group (n=6) and four diabetic groups (n=8).

4.2.3 Research Protocol

After the acclimatization period the experimental animals were assign randomly in 5 groups as follows: 1 healthy control (HC), 1 diabetic control (DC), and 3 groups treated with various doses of *Equisetum arvense* L. extract (50 mg/kg, 100 mg/kg and 200 mg/kg). Following the acclimatization period, the diabetes was induced by intraperitoneal administration of a low dose STZ. Then the animals were treated with the aforementioned horsetail extract doses, for 6 weeks. On week 4 an OGTT, while on week 5 an ITT investigation were performed. At the endpoint of the experiment, following the scarification of animals and prelevation of samples for further investigations, plasma insulin levels and Sirt1 activity were determined (Figure 17.).

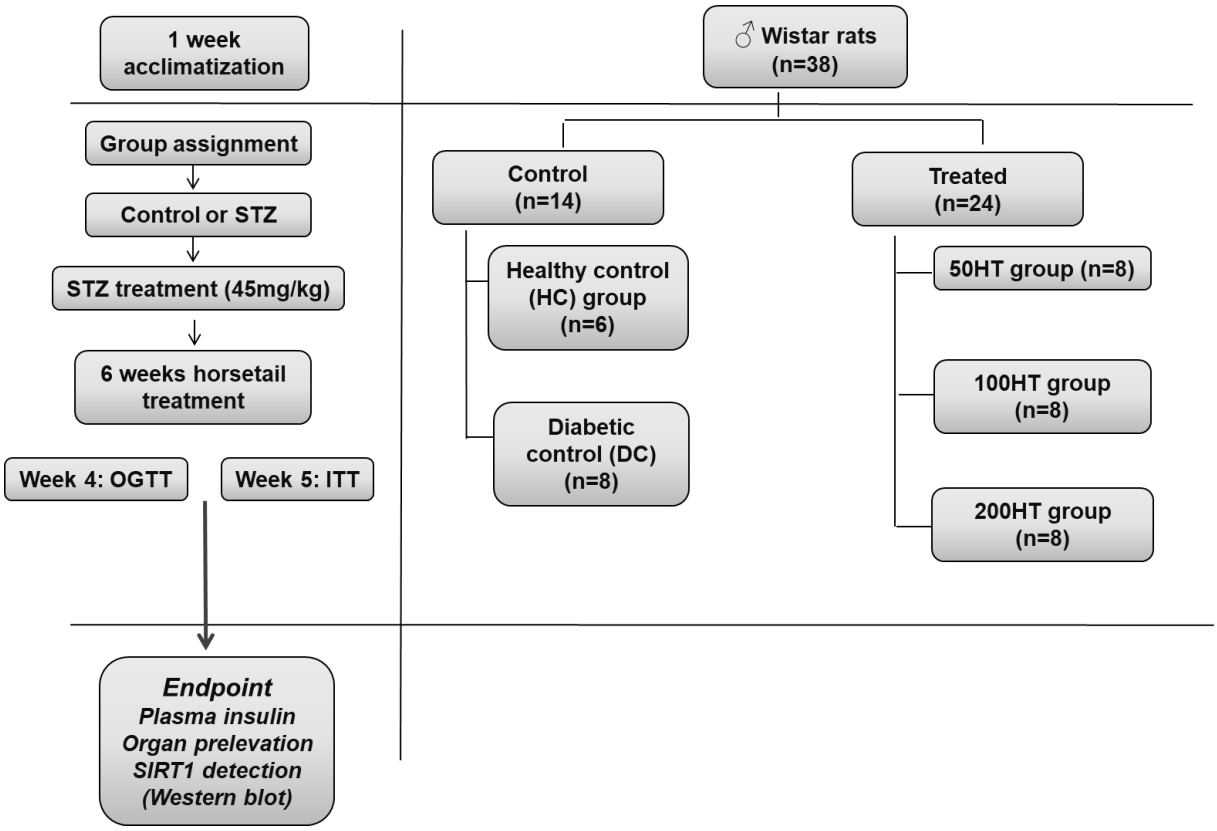

Figure 17. *Research Protocol*

4.2.4 **Induction of Diabetes Mellitus**

To induce diabetes, the diabetic groups were administered an intraperitoneal dose of 45 mg/kg streptozotocin (STZ) obtained from Sigma-Aldrich, Budapest, Hungary. The exact protocol of STZ-induced diabetes mellitus was as follows: blood glucose levels were monitored at 2-hour intervals for 12 hours post-STZ injection. If blood glucose levels exceeded 31 mmol/L, 1 IU insulin was administered via subcutaneous injection. If blood glucose levels surpassed 25 mmol/L, 0.5 IU insulin was administered. Conversely, if blood glucose levels dropped below 2.5 mmol/L, 1 mL of a 40% glucose solution was administered via oral gavage. Additionally, to mitigate the risk of severe hypoglycaemia within the first 24 hours, the animals received a 5% glucose solution. Rats with blood glucose levels exceeding 25 mmol/L five days post-STZ injection were deemed diabetic and retained for further experimentation [253, 254]. Rats exhibiting blood glucose levels below 25 mmol/L were excluded from the study. Following the STZ treatment, the animals were divided into the following groups: healthy control (HC) group (n=6), diabetic control (DC) group treated with vehicle (n=8), 50HT group (n=8), 100HT group (n=8), and 200HT group (n=8). In these groups, the animals were administered 50, 100, or 200

mg/kg of *Equisetum arvense* L. (horsetail) extract, respectively. The extract was dissolved and administered in 1 mL of tap water via oral gavage once daily for six weeks.

4.2.5 Microscopic examination of *Equisetum arvense* L.

For the examination involving *Equisetum arvense* L., microscopical sections of freshly harvested sterile stems were prepared using standard methods [255, 256]. The sections were examined using an OPTIKA B-383PL light microscope (SC Nitech SRL, Bucuresti, Romania) equipped with a 10X objective and a Proview digital camera and software for microscopic analysis.

4.2.6 Preparation of *Equisetum arvense* L. Extract

To prepare the *Equisetum arvense L.* extract, 3.5-4 g of plant material was processed according to the following protocol: 300 mL of 70% ethanol was added to 50 g of horsetail. The mixture was allowed to stand for 24 hours in complete darkness before being filtered. The filtrate was then boiled at 96°C for 120 minutes.

4.2.7 Determination of Phenolic Compounds from *Equisetum arvense* L using UPLC-DAD

To determine the phenolic compounds, present in *Equisetum arvense* L., Ultra-performance liquid chromatography with photodiode array detection (UPLC-DAD) was utilized. The equipment used included a HITACHI Chromaster Ultra RS system (HITACHI, Tokyo, Japan) consisting of a photodiode array detector (model 6430), autosampler (model 6270), interface (model 6310), and pump (model 6170).

UV spectra were recorded at a wavelength of 350 nm. A 10-µL sample was injected, and elution was completed within 15 minutes. Chromatographic conditions were as follows: column - Aquity UPLC BEH Shield RP18, 1.7 µm, 2.1 × 50 mm (Waters); oven temperature - 30°C. The mobile phase consisted of solvent A (0.1% formic acid in water, v/v) and solvent B (100% acetonitrile). The flow rate was set to 0.45 mL/min. The elution gradient was applied as follows: 99% solvent A (0-1 min., isocratic elution), followed by a linear gradient reducing solvent A to 0% over 12 minutes. From 12.5 to 13.5 minutes, the gradient was returned to the initial composition of 99% solvent A, followed by re-equilibration of the column.

4.2.8 Oral Glucose Tolerance Test (OGTT)

On week 4, an oral glucose tolerance test (OGTT) was conducted following the method described by Sunhye Lee et al. with minimal modifications [257]. Prior to the experiment, the

animals underwent an overnight fast, after which basal blood glucose levels were determined via tail clipping. Blood glucose concentration was measured using a glucometer (Accu-Chek, Roche Diagnostics, Budaörs, Hungary). Subsequently, 2 g/kg of glucose was administered via oral gavage, and blood glucose levels were measured at 15, 30, 60, 90, and 120 minutes post-glucose administration. Glucose tolerance was assessed based on the area under the glucose curve.

4.2.9 Insulin Tolerance Test (ITT)

In week 5, an insulin tolerance test (ITT) was conducted according to a method optimized and validated within our institute [258]. Prior to the experiment, the animals underwent a 3-hour fast, followed by determination of basal blood glucose levels via tail clipping. Blood glucose concentration was assessed using a glucometer (Accu-Chek, Roche Diagnostics, Budaörs, Hungary). Subsequently, 0.5 U/kg of insulin was administered intraperitoneally, and blood glucose levels were measured at 30, 60, 90, and 120 minutes post-insulin administration. Insulin tolerance was evaluated based on the area under the glucose curve.

4.2.10 Samples

Following 6 weeks of treatment, the animals were euthanized via cervical dislocation. Left ventricular myocardial tissue, as well as epididymal and retroperitoneal adipose tissue, were excised and stored at −80°C for subsequent analysis. Adiposity was quantified as the sum weight of retroperitoneal and epididymal WAT, normalized to body weight.

4.2.11 Plasma insulin concentration

Plasma insulin levels were determined using a commercially available insulin radioimmunoassay (RIA) kit (RK 400 M, Institute of Isotopes Budapest, Hungary). Both intra- and inter-assay variations were found to be lower than 5%.

4.2.12 Western Blot

For the analysis and detection of SIRT1 from left ventricle tissues of animals, we utilized Western blot technique. Tissue samples were homogenized in a buffer containing Tris (25 mM), NaCl (25 mM), Na-orthovanadate (1 mM), NaF (10 mM), Na-pyrophosphate (10 mM), okadaic acid (10 nM), EDTA (0.5 mM), PMSF (1 mM), protease inhibitor cocktail, and distilled water (all from Sigma-Aldrich, St. Louis, MO, USA) using a homogenizer (IKA-WERKE, Staufen, Germany). The total protein concentration was determined using an automated

spectrophotometer (FLUOstar Optima, BMG Labtech, Ortenberg, Germany) and a bicinchoninic acid (BCA) assay kit (Sigma-Aldrich, St. Louis, MO, USA).

Fifty micrograms of total protein per well, including samples from the nuclear fraction and protein standards, were electrophoretically separated using 12% SDS-polyacrylamide gels at 25 mA for 120–150 minutes. The fractionated proteins were then transferred onto nitrocellulose membranes. The membranes were blocked in Tris-buffered saline with Tween 20 (TBS-T) containing 3% bovine serum albumin (BSA) for 1.5 hours. Subsequently, each blot was incubated overnight at 4°C with anti-SIRT1 antibodies (Abcam Plc., Cambridge, UK), diluted to 1:1000 in TBS-T, followed by incubation with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich-Merck KGaA, Darmstadt, Germany). Histone H3 (15 kDa) was utilized as a nuclear housekeeping internal control.

Enhanced chemiluminescent substrate (WesternBright™, ECL, Advansta Inc., Menlo Park, CA, USA) was applied to identify bands corresponding to SIRT1 and Histone H3 proteins. Detection and analysis were performed using a C-Digit® blot scanner with Image Studio Digits ver. 5.2. Software (LI-COR Inc., Lincoln, NE, USA). Data were averaged from two independent experiments (n = 4/group).

4.2.13 **Statistics**

The data were expressed as mean ± standard error of the mean (SEM). Analysis of blood glucose and body weight was conducted using two-way analysis of variance (ANOVA), while additional data were subjected to one-way ANOVA followed by a modified t-test for repeated measures based on Tukey's method.

5 Results

5.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats

5.1.1 The effects of diosgenin and fenugreek seed treatment on body weight

As it shown in Figure 18. the animals administered with 1 mg/kg diosgenin and 0.6 g/kg fenugreek seeds exhibited a notable increase starting from day 4 in comparison to the healthy controls, and this significant difference persisted throughout the experimental duration [258].

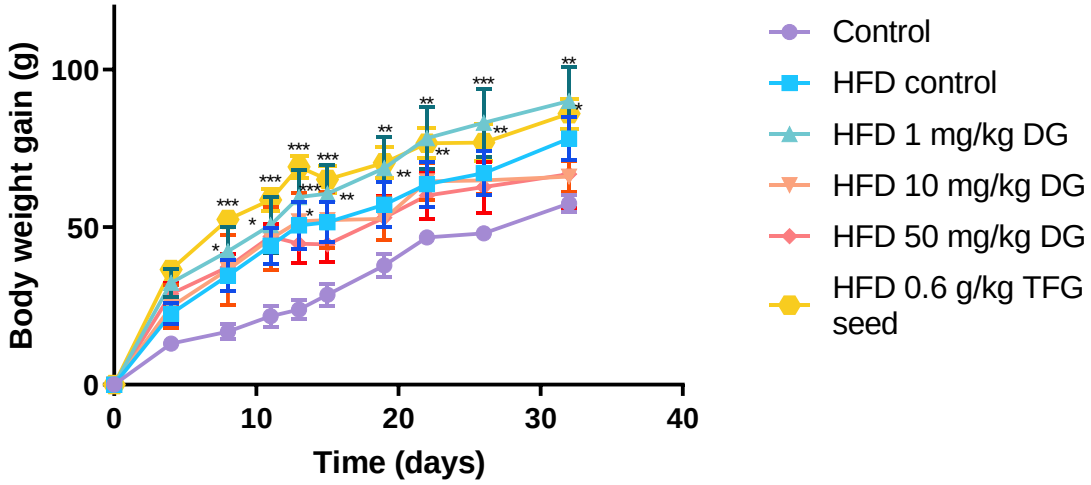

Figure 18. *Effect of chronic diosgenin and fenugreek seed treatment on body weight gain. The *, ** and *** indicates significant difference compared to the control group ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).*

5.1.2 Effect of chronic diosgenin and fenugreek seed treatment on the weight of abdominal white adipose tissue

A diet rich in fat and sugar led to a significant difference in WAT accumulation. However, there was no observable dose-response relationship among the various doses of diosgenin concerning adipose tissue weight, even with the HFD 10 mg/kg DG group, which exhibited the most significant weight gain compared to healthy controls. Conversely, fenugreek resulted in a significant increase compared to the control group, as depicted in Figure 16. While these effects were significant compared to the control group, they did not reach significance compared to the HFD control group. Interestingly, we observed that at high dosages, diosgenin and TFG showed a tendency to decrease WAT accumulation (Figure 19). Conversely, low dosages of diosgenin and TFG seeds were insufficient to counteract the effects of HFD [258].

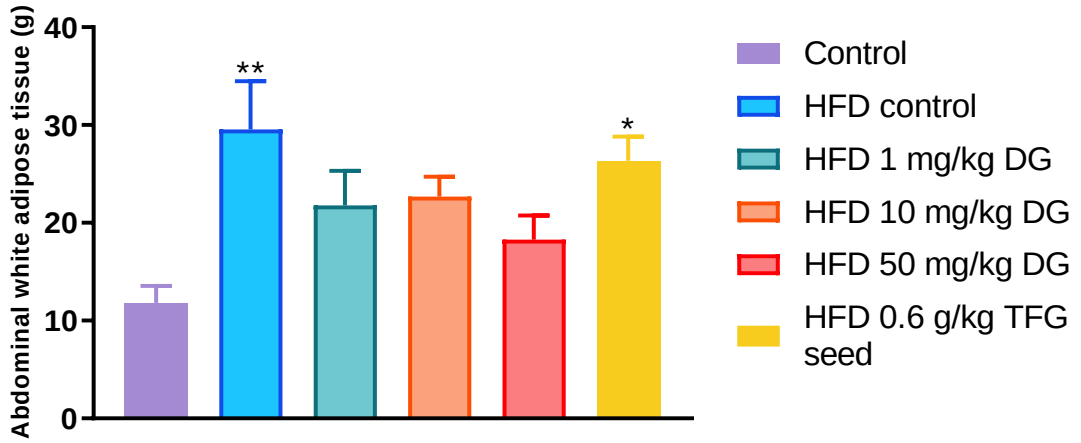

Figure 19. *Effect of chronic diosgenin and fenugreek seed treatment on the weight of abdominal white adipose tissue. The * and ** indicates significant difference compared to the control group ($p<0.05$ and $p<0.01$, respectively).*

5.1.3 Effect of chronic diosgenin and fenugreek seed treatment on daily food intake

Figure 20. illustrates the food consumption throughout the treatment period. It was observed that all HFD groups consumed significantly less food compared to the control group [258].

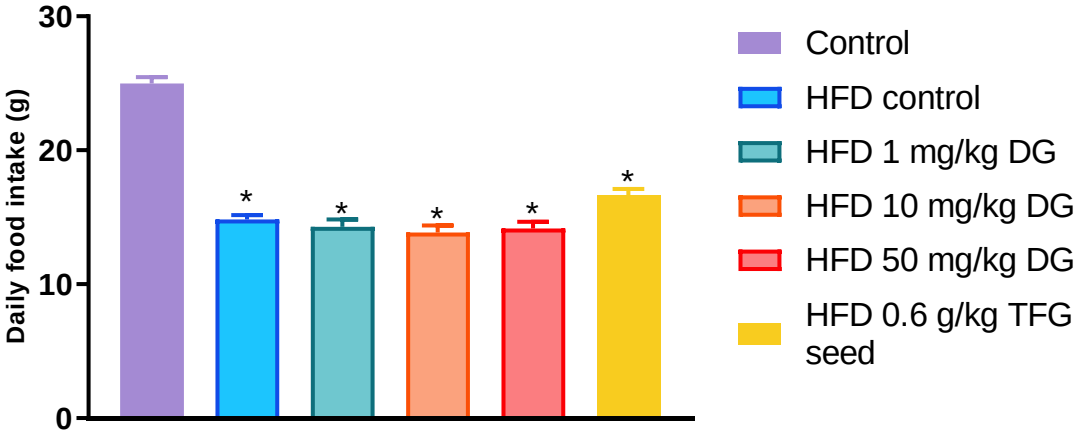

Figure 20. *Effect of chronic diosgenin and fenugreek seed treatment on daily food intake. The * indicates significant difference compared to the control group ($p<0.05$)[258].*

5.1.4 Effect of chronic diosgenin and fenugreek seed treatment on daily water intake

Figure 21. illustrates water consumption during the treatment period. There was a significant increase in water consumption observed in the groups treated with 10 mg/kg and 50 mg/kg of diosgenin and fenugreek [258].

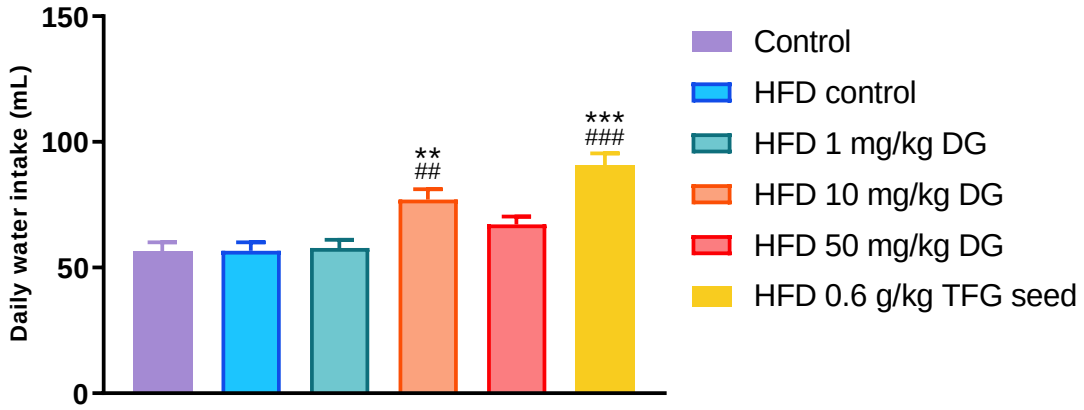

Figure 21. *Effect of chronic diosgenin and fenugreek seed treatment on daily water intake. The ** and *** indicates significant difference compared to the control group ($p<0.01$ and $p<0.001$, respectively). The ## and ### indicates significant difference compared to the HFD control group ($p<0.01$ and $p<0.001$, respectively).*

5.1.5 Effect of chronic diosgenin and fenugreek seed treatment on daily energy intake

We observed that both the HFD control group and the diosgenin-treated group consumed a similar amount of calories per day compared to the control group. However, the energy intake of the fenugreek-treated rats was significantly higher compared to both the HFD control and diosgenin-treated groups, as demonstrated in Figure 22 [258].

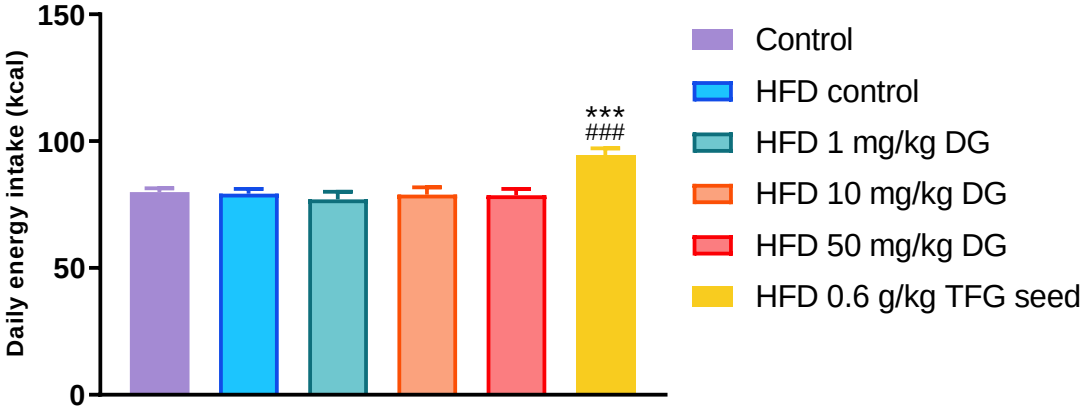

Figure 22. *Effect of chronic diosgenin and fenugreek seed treatment on daily energy intake. The *** indicates significant difference compared to the control group ($p<0.001$). The ### indicates significant difference compared to the HFD control group ($p<0.001$).*

5.1.6 Effect of chronic diosgenin and fenugreek seed treatment on insulin sensitivity

During the insulin tolerance test, we observed no significant difference in the area under the curve between the groups, as depicted in Figure 23. and Figure 24. These findings suggest that

there was no variance in insulin sensitivity among the groups, as assessed by the area under the curve during the insulin tolerance test [258].

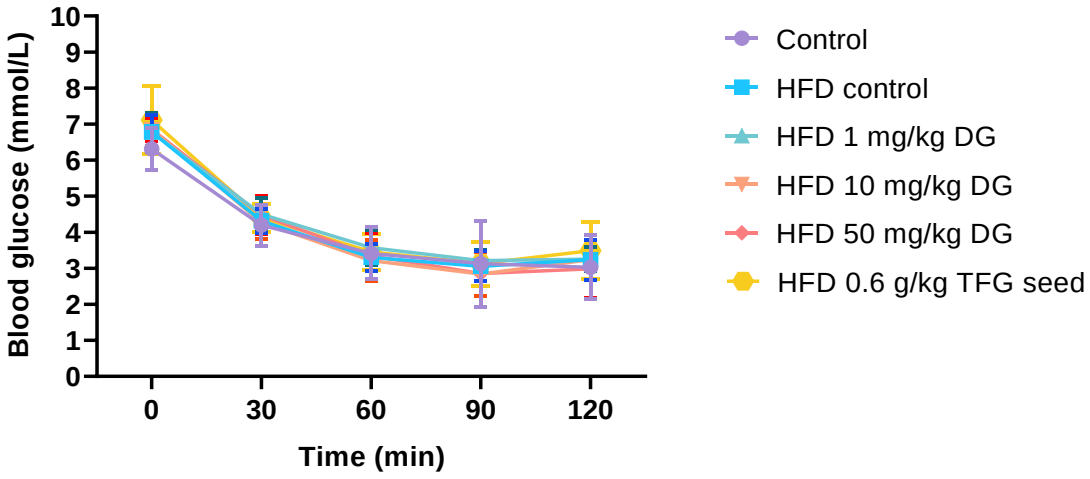

Figure 23. *Effect of chronic diosgenin and fenugreek seed treatment on blood glucose levels during ITT.*

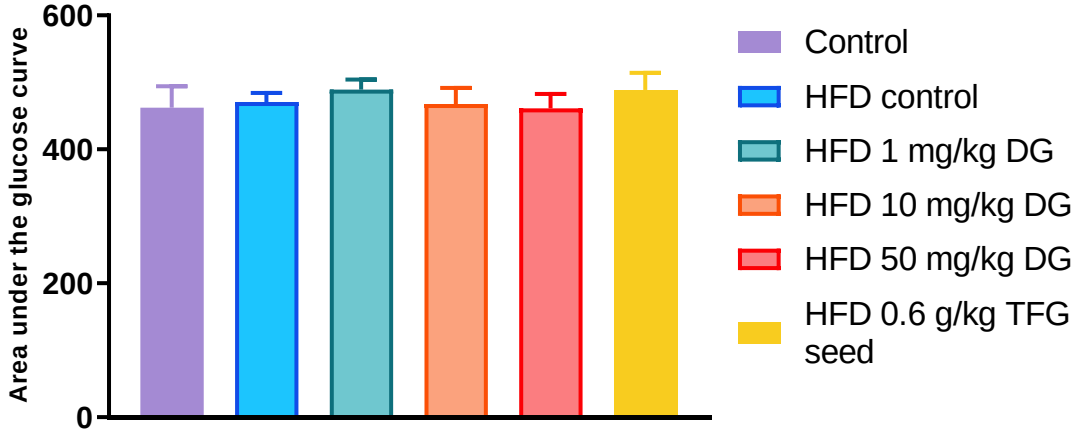

Figure 24. *Effect of chronic diosgenin and fenugreek seed treatment on area under the glucose curve during ITT.*

5.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats

5.2.1 Microscopic Examination of *Equisetum arvense* L.

Using microscopic analysis to distinguish *Equisetum arvense* L. from other species containing potentially toxic alkaloids, we provide the following description of the sterile stems. These stems exhibit 6–18 edges or ridges. Beneath the silicified chlorenchyma tissue on the edges, assimilated palisade forms the cortical parenchyma. Within the parenchyma, vallecular canals are present, situated within expansive aeriferous areas and taking various forms such as channels, gaps, and circles, all organized systematically. The central cylinder initiates with a pericycle, characterized by small, closely packed cells. Within the fundamental parenchyma of the pith, numerous vascular bundles are arranged in a ring formation. In each vascular bundle, the phloem tissue lies just beneath the pericycle and is more developed than the surrounding xylem tissue, which encircles the carinal canal filled with water (refer to Figure 25.). The stem's branches exhibit four distinct edges, with silicified chlorenchyma on the upper surface and well-developed assimilated palisade parenchyma beneath. Consistent with literature findings, the central cylinder displays four vascular bundles, devoid of a carinal canal (F.R.X., Ph.Hg. VIII., Eur.Ph. 7th Edition) [255, 256, 259] (Figure 26.).

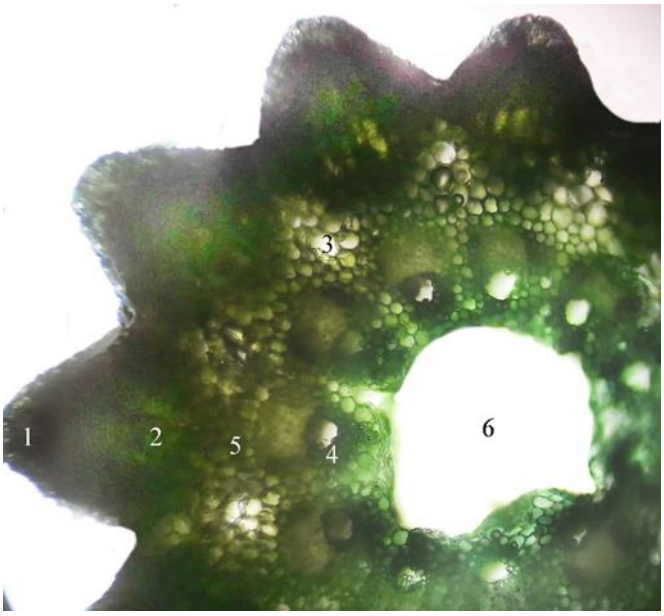

Figure 25. *Equisetum arvense* L. *main stem cross section (100X): 1—chlorenchyma; 2—assimilated palisade parenchyma; 3—aeriferous areas in the ground tissue, known as vallecular canals; 4—vascular bundles; 5—cortical parenchyma; 6—pith cavity.*

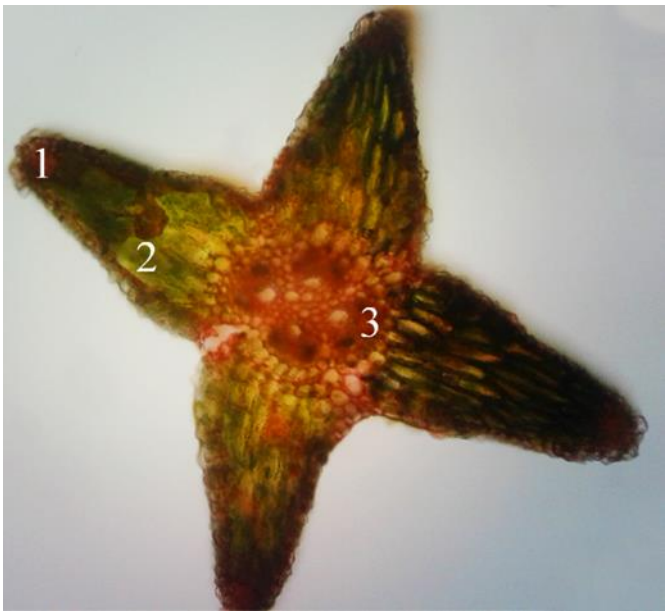

Figure 26. *Equisetum arvense* L. *stem ramification cross section (100X). 1—silicified chlorenchyma; 2—palisade parenchyma; 3—leading bundle.*

5.2.2 Ultra-High Performance Liquid Chromatography (UHPLC) Analysis of *Equisetum arvense* L. Extract

To determine the concentration of phenolic compounds, present in the *Equisetum arvense* L. extract, we conducted UHPLC analysis. The results of the chromatography (recorded at 350nm) are shown in Figure 27 [260].

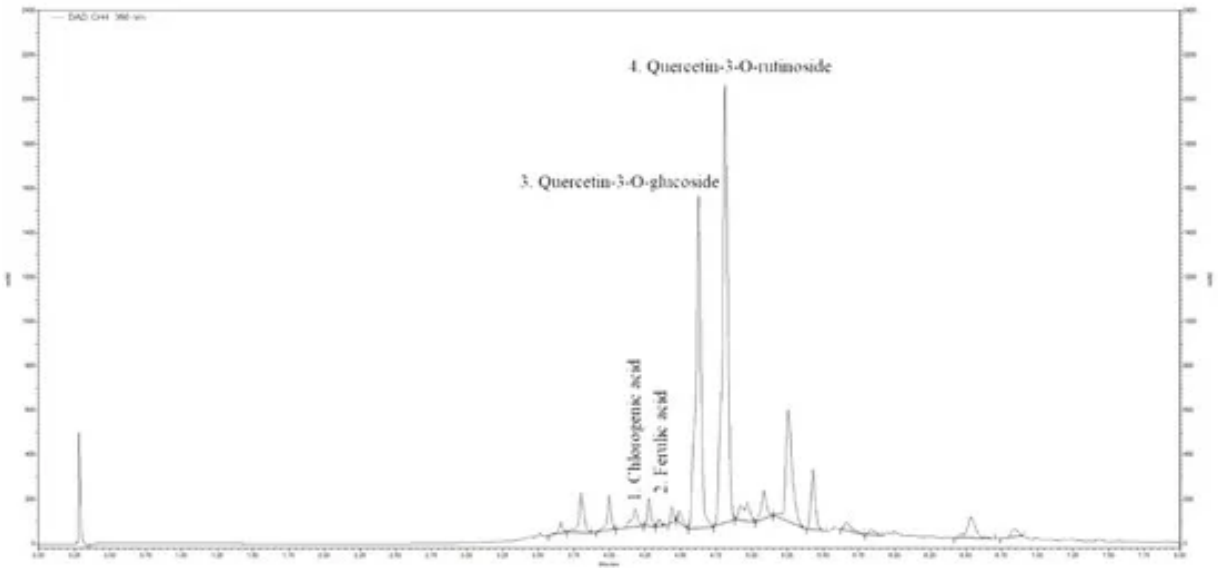

Figure 27. *Ultra-High Performance Liquid Chromatography (UHPLC) chromatograms of the Equisetum arvense* L. *extract at 350 nm. Confirmed by standard.*

The predominant flavonoids identified in these analyses are presented in Table IX. Our findings revealed that 1 g dry weight of *Equisetum arvense* L. contains 63.65 µg of quercetin, of which 27.13 µg of quercetin-3-O-glucoside and 36.52 µg of quercetin-3-O-rutinoside. Consequently,

the quercetin concentration in 1g of *Equisetum arvense* L. extract used in our experimental protocol was 795.625 µg. The doses of applied *Equisetum arvense* L. extract contained the following amounts of quercetin: 50 mg/kg extract contained 39.78 µg, 100 mg/kg contained 79.56 µg, and 200 mg/kg contained 159.3 µg [260].

Table IX. *Phenolic compound concentrations in the Equisetum arvense L. extract.*

No	RT (min)	Compound	Concentration of Phenolic Compounds (µg g^{-1} Dry Mass)
1	4.18	Chlorogenic acid	1.735
2	4.35	Ferulic acid	0.355
3	4.624	Quercetin-3-*O*-glucoside	27.13
4	4.810	Quercetin-3-*O*-rutinoside	36.52

5.2.3 Effect of *Equisetum arvense* L. on Body Weight

For the investigation of above mentioned effect we conducted biweekly body weight measurements to assess whether *Equisetum arvense* L. treatment could impact the weight gain associated with streptozotocin (STZ) treatment. The findings revealed that at the study's outset, there were no statistically significant differences in body weights among the groups (Figure 28). Following the STZ treatment, the diabetic groups exhibited a gradual decrease in weight gain compared to the healthy rats. Specifically, the Diabetic Control (DC) animals displayed significantly reduced body weight from day 3 onward, while the *Equisetum arvense* L. treated animals exhibited significantly lower weights from day 6 onward compared to the Healthy Control (HC) rats. Notably, the 100HT (treatment with 100 mg/kg *Equisetum arvense* L. extract) and 200HT (treatment with 200 mg/kg *Equisetum arvense* L. extract) groups demonstrated a significant increase in body weight compared to the DC animals from day 17 until the end of the experiment [260].

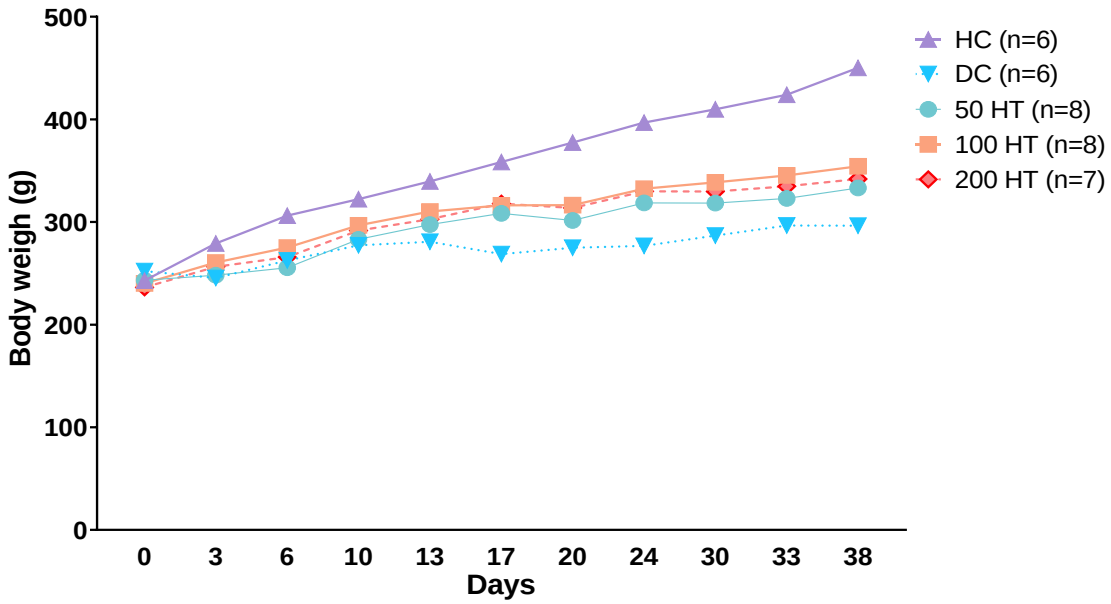

Figure 28. *Effect of Equisetum arvense* L. *on body weight. The experimental groups names were abbreviated, as follows: healthy control (HC), diabetic control (DC), 50HT (animals treated with 50 mg/kg Equisetum arvense* L. *extract), 100HT (animals treated with 50 mg/kg Equisetum arvense* L. *extract) and 200HT (animals treated with 50 mg/kg Equisetum arvense* L. *extract).*

5.2.4 Effect of *Equisetum arvense* L. on Blood Glucose

In our investigation, we conducted daily blood glucose measurements to assess whether treatment with *Equisetum arvense* L. extract could reduce blood glucose levels associated with STZ treatment. The results of the morning blood glucose readings are presented in Figure 29. Following induction of diabetes with STZ, blood glucose levels significantly increased from one day after induction in the STZ-treated groups. Throughout the six-week period, blood glucose levels remained relatively stable within the different treatment groups; however, animals treated with *Equisetum arvense* L. extract exhibited a statistically significant improvement compared to the DC rats [260].

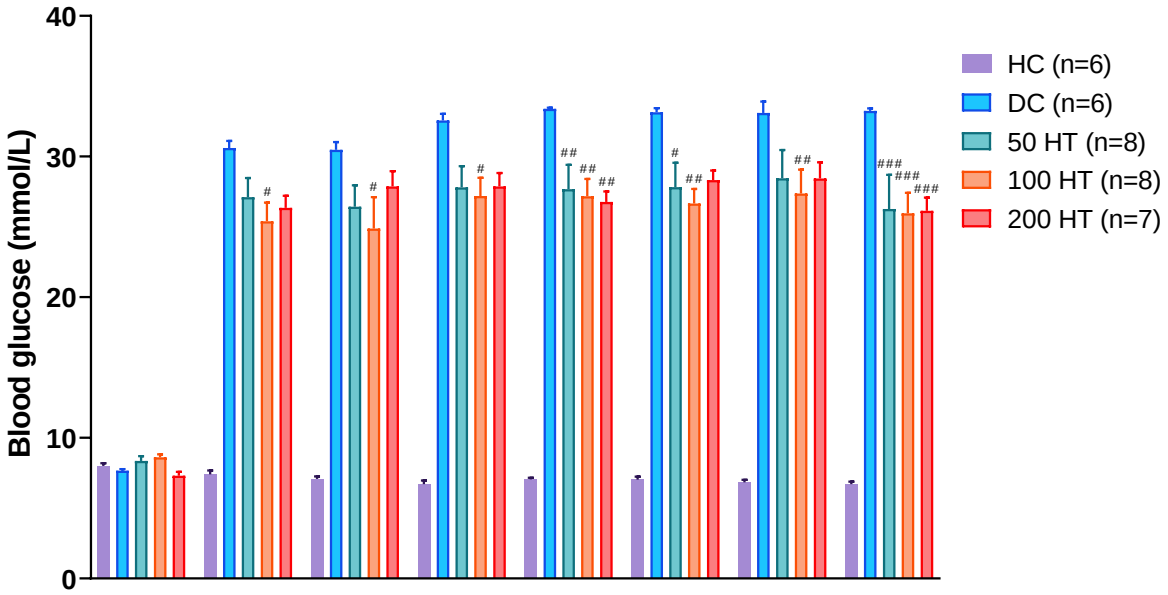

Figure 29. *Effect of Equisetum arvense* L. *on blood glucose. From day 1, all groups showed a significant difference from the HC group ($p<0.001$). For clearer representability it is not shown with symbols. The #, ##, and ### indicates significant difference from the DC group ($p<0.05$, $p<0.01$, and $p<0.001$, respectively).*

5.2.5 Effect of *Equisetum arvense* L. on Glucose Tolerance

To assess the impact of *Equisetum arvense* L. on glucose intolerance associated with STZ treatment, we conducted an oral glucose tolerance test (OGTT) in week 4. The area under the glucose curve was markedly increased in all STZ-treated groups (Figure 30.B), indicating reduced glucose tolerance. However, a statistically significant improvement was observed only in the 100HT group compared to both the DC and *Equisetum arvense* L. treated animals. For better visualization of the data, a time-dependent graph was utilized (Figure 30.A) [260].

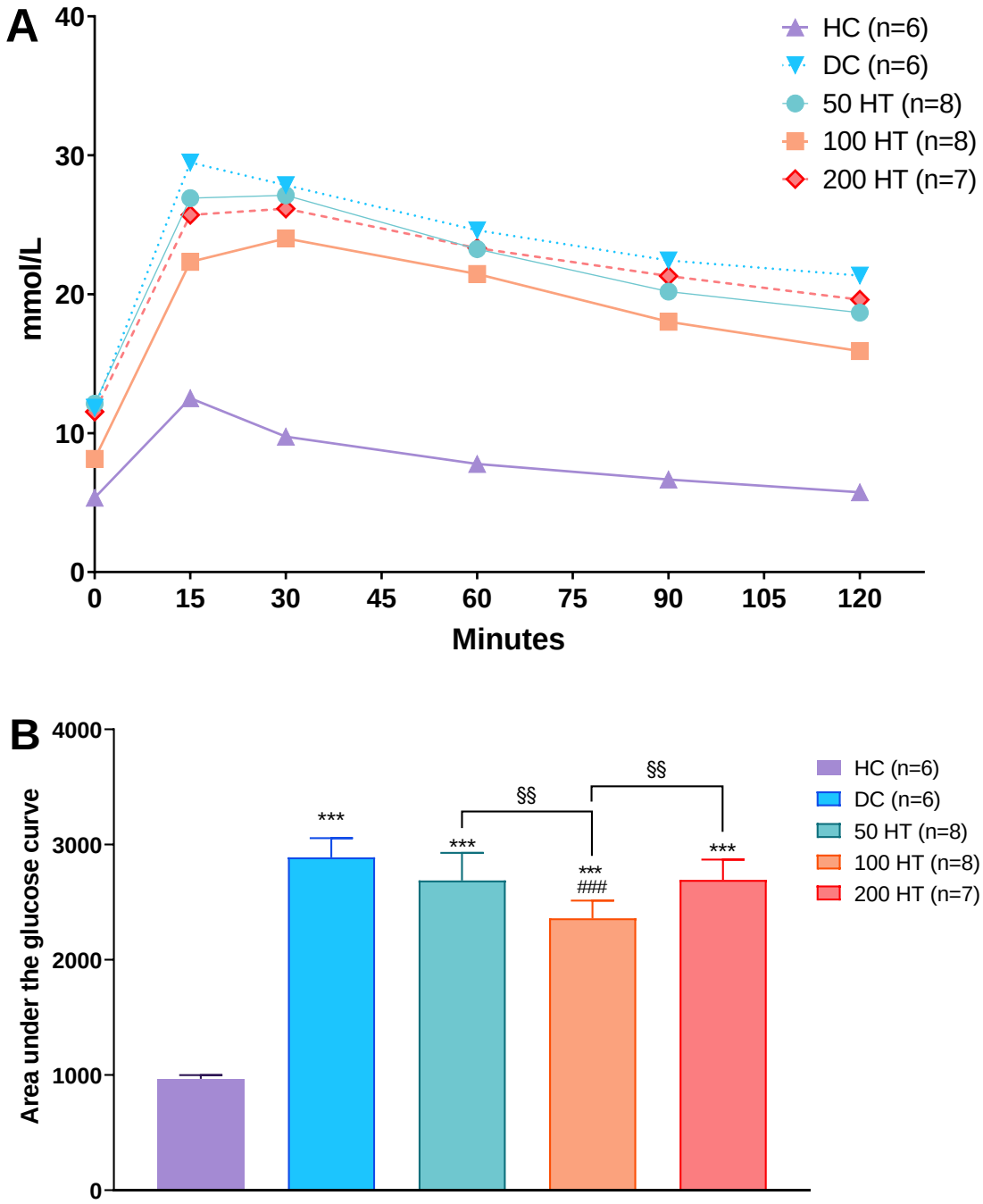

Figure 30. *Effect of Equisetum arvense* L. *on blood glucose levels (**A**) and area under the glucose curve during oral glucose tolerance test (**B**). The *** indicates significant difference from the HC group (p<0.001, respectively). The ### indicates significant difference from the DC group (p<0.001, respectively). The §§ indicates significant difference between the connected groups (p<0.01).*

5.2.6 Effect of *Equisetum arvense* L. on Insulin Tolerance

To assess the impact of *Equisetum arvense* L. on impaired insulin response, we conducted an insulin tolerance test (ITT) in week 5. The area under the glucose curve was significantly elevated in all STZ-treated groups (Figure 31.B), indicating decreased glucose tolerance. However, a notable improvement was observed only in the 100HT group. The effect of

Equisetum arvense L. extract on blood glucose levels measured during the ITT is illustrated in Figure 31.A [260].

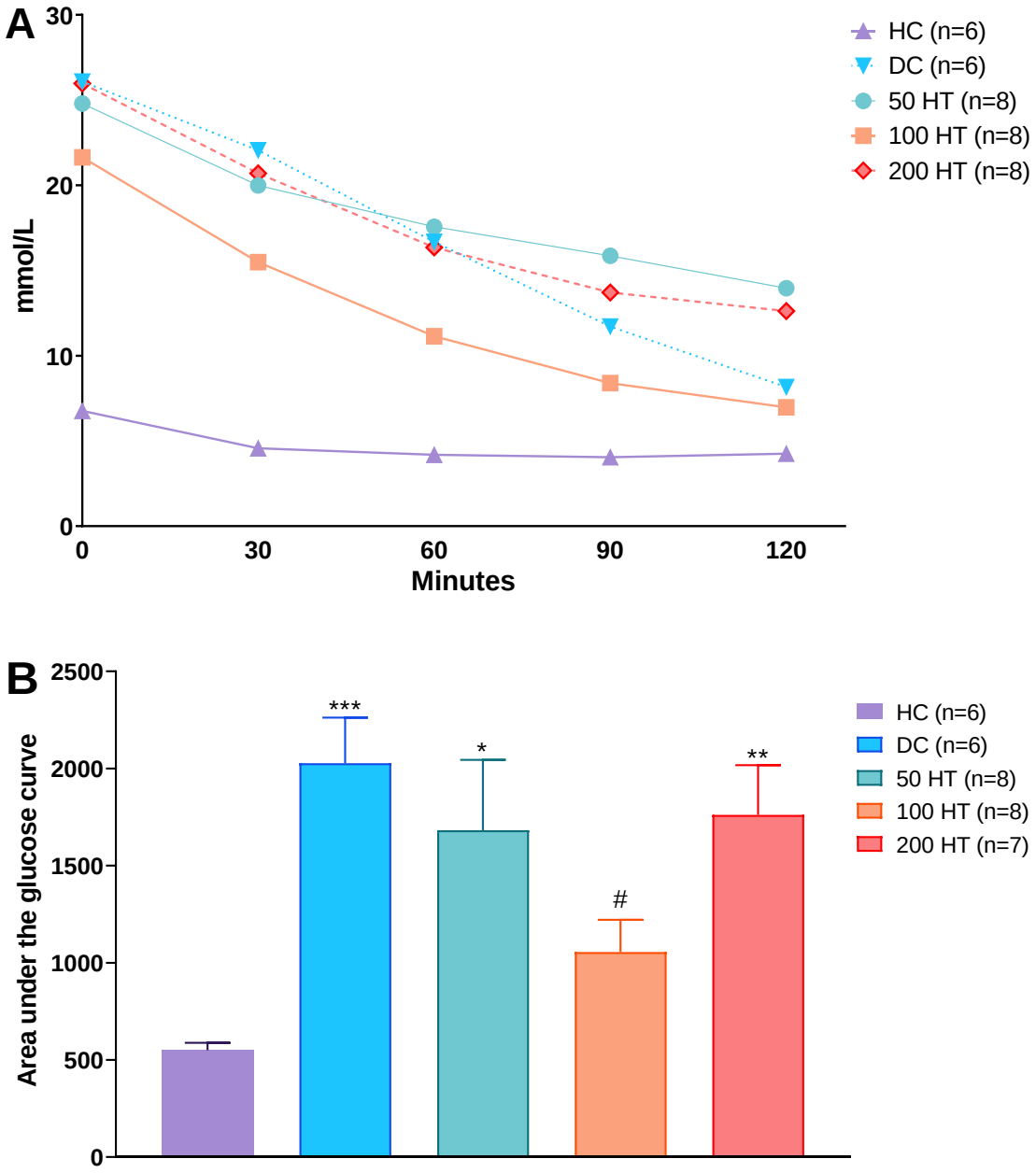

Figure 31. *Effect of Equisetum arvense* L. *on blood glucose levels (**A**) and area under the glucose curve during insulin tolerance test (**B**). The *, **, and *** indicates significant difference from the HC group ($p<0.05$, $p<0.01$, and $p<0.001$, respectively). The # indicates significant difference from the DC group ($p<0.05$).*

5.2.7 Effect of *Equisetum arvense* L. on Fasting Plasma Insulin

To assess whether *Equisetum arvense* L. could ameliorate impaired insulin production linked to STZ treatment, we measured fasting plasma insulin levels from the blood samples collected at the end of the study. However, all STZ-treated groups exhibited significantly reduced plasma

insulin levels (induced by STZ therapy) compared to the HC group, it was observed that the pancreatic β-cell function was partially preserved. (Figure 32.) [260].

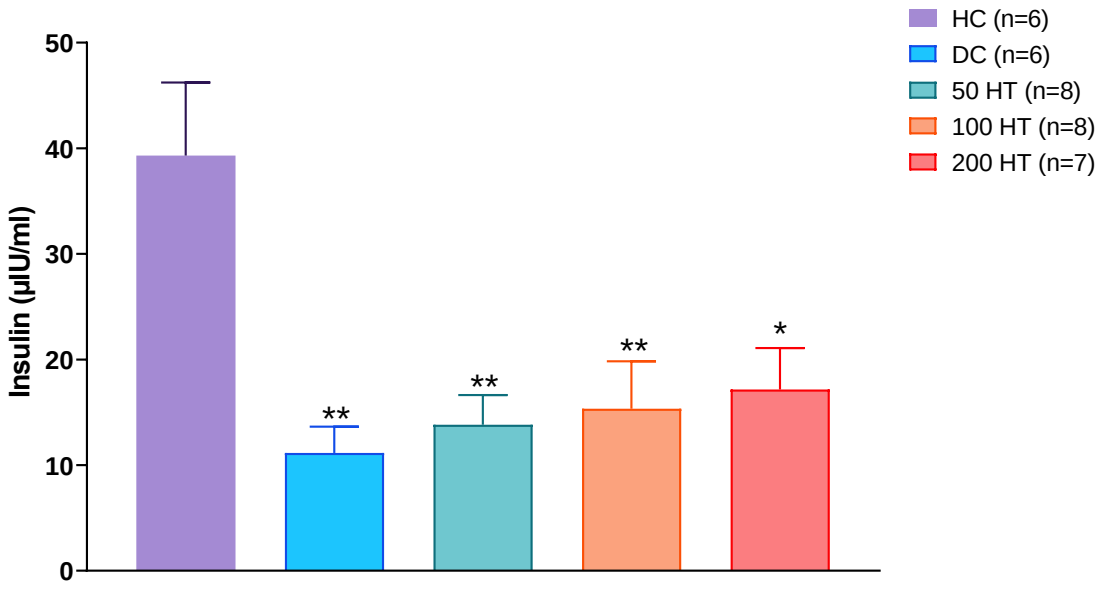

Figure 32. *Effect of Equisetum arvense* L. *on fasting plasma insulin. The * and ** indicates significant difference from HC group (p<0.05 and p<0.01, respectively).*

5.2.8 Effect of *Equisetum arvense* L. on Adiposity

To evaluate the potential impact of *Equisetum arvense* L. on adipose tissue development, we assessed adiposity at the study's endpoint by measuring the combined weight of retroperitoneal and epididymal WAT, normalized to body weight (Figure 33.). At the end of the experimental period, all diabetic groups exhibited a statistically significant reduction in fat mass. However, treatment with *Equisetum arvense* L. showed no effect on this parameter [260].

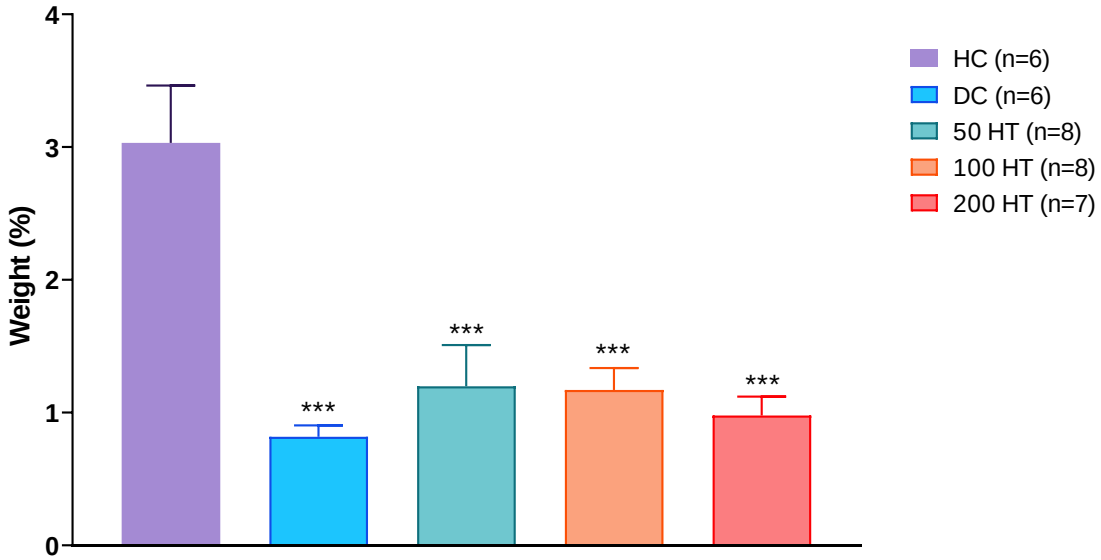

Figure 33. *Effect of Equisetum arvense* L. *on adiposity. The *** indicates significant difference from the HC group (p<0.001).*

5.2.9 Effect of *Equisetum arvense* L. on Heart Weight Index

Following the collection of animal tissues, the mass of insulin-sensitive tissues including the heart, liver, and abdominal WAT was assessed. We noted that the hearts of STZ-treated animals exhibited elevated values compared to the healthy control group. Consequently, we calculated the heart index, which represents the heart weight normalized to the body weight of the animals, and subjected the results to statistical analysis (Figure 34.). A statistically significant increase in heart weight index was observed in the DC and 200HT groups [260].

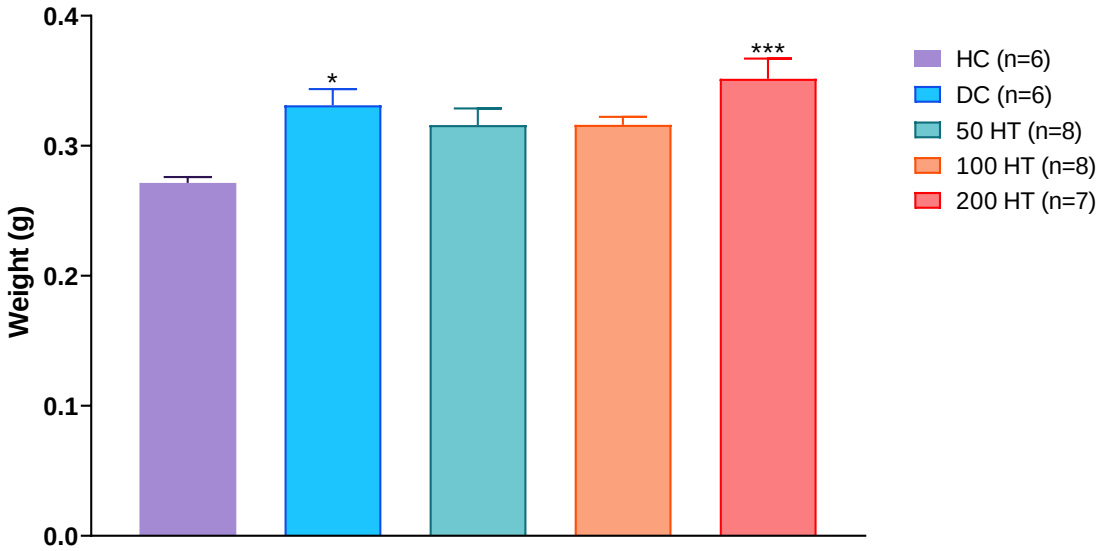

Figure 34. *Effect of Equisetum arvense* L. *on heart weight index. The * and *** indicates significant difference from the HC group (p<0.05 and p<0.001, respectively).*

5.2.10 Effect of *Equisetum arvense* L. on SIRT1 Levels

Our study aimed to explore the involvement of sirtuins, specifically SIRT1, in the pathogenesis of diabetes and diabetic cardiomyopathy, and to examine the potential effects of *Equisetum arvense* L. extract in these pathological conditions. Western blot analysis was utilized to assess SIRT1 protein expression in cardiac tissue, with results depicted in (Figure 35). Normalized to the Histone H3 housekeeping protein, a notable decrease was observed in all STZ-treated groups compared to the healthy control (HC). Statistical analysis revealed similar decreasing trends in the DC and 50HT groups ($p<0.01$), while the 100HT group exhibited a significant difference ($p<0.05$) compared to the HC group. Remarkably, the highest dose of *Equisetum arvense* L. extract (200HT) led to the weakest SIRT1 protein expression ($p<0.001$). Consequently, we speculate that the 100 mg/kg dose of *Equisetum arvense* L. extract may activate SIRT1 and interfere with signal pathways involving SIRT1 activity; however, the

duration of treatment proved insufficient to yield a significant effect compared to the diabetic control [260].

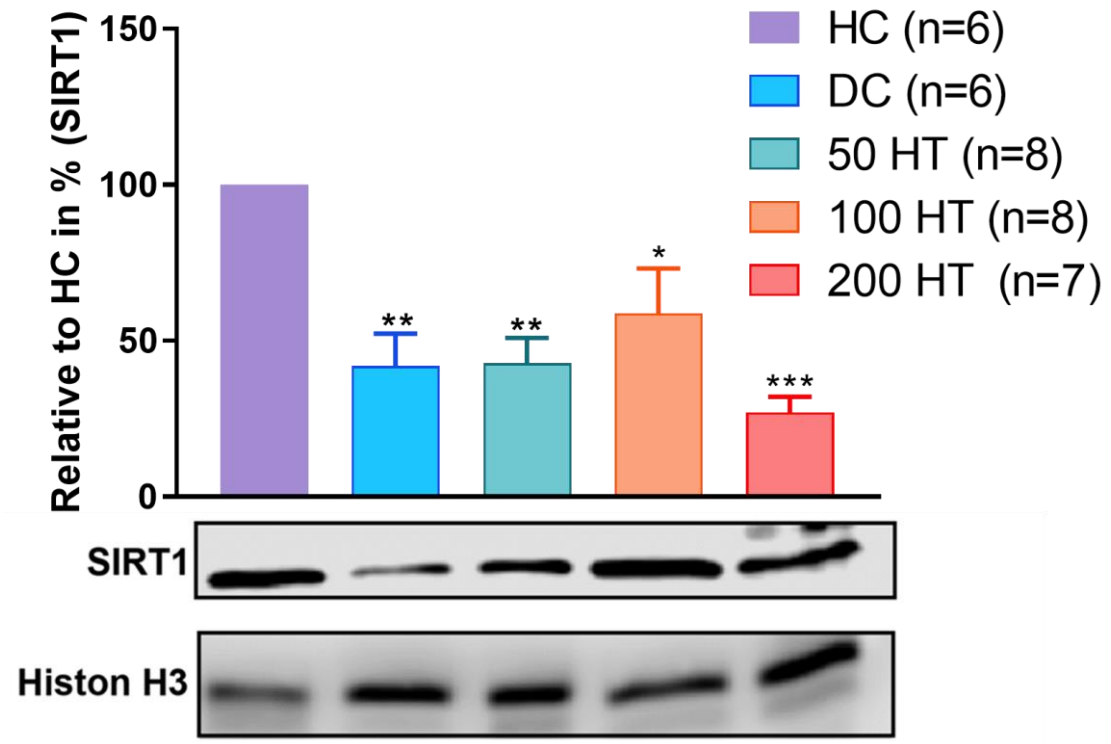

Figure 35. *Effect of Equisetum arvense* L. *on SIRT1 protein expression in left ventricle cardiac tissue. The *, **, and *** indicates significant difference from HC group ($p<0.05$, $p<0.01$, and $p<0.001$, respectively).*

6 Discussion

6.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats

TFG, while not intended as a weight loss aid, is commonly employed as a nutritional supplement in the management of diabetes mellitus due to its capacity to normalize blood glucose levels and enhance insulin sensitivity. However, our study revealed that when combined with a western diet at low doses, TFG may elevate the risk of weight gain or obesity. This novel finding highlights a potentially significant side effect of TFG, underscoring the importance of careful dosing for patients using TFG as a food supplement or for physicians prescribing it to mitigate the potential adverse impact on body weight.

A high-fat and high-sugar diet led to a notable increase in WAT accumulation. However, there was no apparent dose-response relationship observed with different doses of diosgenin in terms of adipose tissue weight, even with the HFD 1 mg/kg DG group, which exhibited the greatest weight gain compared to healthy controls. Conversely, fenugreek resulted in a significant increase compared to the control group. Interestingly, higher doses of diosgenin and TFG exhibited a tendency to reduce WAT accumulation. Conversely, lower doses of diosgenin and TFG seed failed to offset the effects of the HFD. Additionally, TFG seeds, aside from promoting obesity, also elevated abdominal WAT, which correlates with the onset of diabetes mellitus. Although a HFD obviously contains more calories than standard chow, the inclusion of a sucrose solution in the HFD groups introduced additional calories to daily energy intake. Notably, while the HFD control and diosgenin-treated groups consumed similar amounts of calories per day compared to the control group, the fenugreek-treated rats exhibited a significantly higher energy intake compared to both the HFD control and diosgenin-treated groups.

Our findings reveal that despite diet-induced obese rats reducing food intake to counterbalance the energy-rich diet, they exhibited accelerated body weight gain. Additionally, HFD controls demonstrated a significant elevation in abdominal white adipose tissue (WAT) weight. Diosgenin treatment alone failed to mitigate the adverse effects of the high-fat diet on body weight, adiposity, and energy metabolism. Conversely, chronic treatment with fenugreek seed exacerbated these parameters, suggesting that other bioactive compounds present in TFG, rather than diosgenin, are responsible for the metabolic alterations. Recent research has highlighted the role of 4-OH isoleucine or galactomannan in fenugreek seeds' glucose tolerance-improving

and insulin-sensitizing effects [261-264]. The observed effect of fenugreek on body weight gain may be linked to its ability to modulate hormones in the central nervous system involved in food intake regulation, such as melanin-concentrating hormone (MCH). Fenugreek acts as an MCH agonist, as supported by previous studies [10, 265-271].

Furthermore, our findings indicate that despite TFG enhancing weight gain and increasing abdominal adiposity – parameters closely associated with the development of IR – six weeks of treatment with fenugreek seeds did not alter insulin sensitivity. It is widely recognized that fenugreek exhibits an insulin-sensitizing effect and delays the onset of glucose intolerance in susceptible individuals [261, 264, 270, 272-274]. However, our results with diosgenin failed to validate this, suggesting that sapogenins may not significantly contribute to the insulin-sensitizing effect of TFG. Additionally, the administered dose of TFG may be insufficient to enhance insulin sensitivity in the DIO model.

Chronic administration of fenugreek led to an increase in body weight gain induced by a diet rich in fat and sugar. Moreover, abdominal adiposity and calorie consumption were elevated in the TFG-treated group compared to control and DIO control animals. However, despite its adverse effects on body weight, abdominal fat, and energy intake, fenugreek treatment did not negatively impact insulin sensitivity of peripheral tissues. Our experimental protocol demonstrated that lower doses of diosgenin and an appropriate dose of TFG in combination with a high-calorie diet can elevate the risk of obesity. These findings are particularly relevant for patients using TFG as a nutritional supplement to normalize blood glucose levels, either alone or in combination with specific antidiabetic therapy.

Despite the documented insulin-sensitizing properties of TFG in the literature, we were unable to replicate this effect in our study, likely due to the relatively modest dosage utilized in our research. We came to the conclusion that diosgenin alone does not induce significant body weight or fat accumulation. However, it is probable that diosgenin interacts in a multifaceted manner with other constituents of fenugreek seeds, potentially exhibiting synergistic effects. Nonetheless, further investigations are warranted to elucidate the underlying mechanisms and the contributions of individual active ingredients.

6.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats

Diabetes mellitus stands as the foremost prevalent metabolic disorder globally, with a trajectory of escalating incidence and prevalence. Characterized by persistent hyperglycaemia often concomitant with IR, diabetes mellitus precipitates complications such as neuropathy, arteriopathy, renal dysfunction, and cardiomyopathy. Despite the introduction of novel classes of antidiabetic agents into clinical practice in recent years, the therapy for diabetes mellitus remains incompletely resolved [275-277]. Hence, ongoing extensive research aims to explore novel signalling pathways implicated in the pathogenesis of diabetes mellitus. Such investigations seek to foster the development of more advanced and efficacious antidiabetic agents, as well as strategies to potentially mitigate or retard the disease progression and ameliorate its associated complications [278-281].

Medicinal herbs, notably those recognized for their antidiabetic properties such as fenugreek, cinnamon, sage, berries, and turmeric, are garnering increasing attention as primary or adjunctive therapeutic agents for diabetes management [282-284]. Field horsetail (*Equisetum arvense* L.) stands among the most widely used herbs, with a medicinal history dating back to ancient times. Its enduring popularity persists due to its ubiquitous presence worldwide and versatile applications. Field horsetail is full with diverse active compounds, notably polyphenols, flavonoids, saponins, dietary fibres, vitamins A, E, and C, potassium, calcium, and silicates. With its array of bioactive constituents, horsetail finds utility across various medical domains. It is administered internally and/or topically to arrest nasal, pulmonary, or gastric haemorrhages, serve as a diuretic, and treat ulcers, rheumatoid arthritis, and slow-healing wounds.

Ancient and contemporary records from Asia also acknowledge the antidiabetic potential of horsetail [223, 224]. Thus, we selected this botanical specimen as the focal point of our investigation to assess its capacity to ameliorate blood glucose alterations and potentially mitigate symptoms associated with prediabetes and diabetes, such as IR. Our hypothesis assumed that due to the presence of flavonoids and other bioactive constituents, field horsetail could exert beneficial effects potentially mediated by the upregulation of SIRT1 expression. Our experimental findings revealed distinct effects of ethanol extract of horsetail across varying doses. Notably, fasting plasma insulin levels remained low yet detectable in STZ-treated rats, indicative of a diabetic phenotype characterized by inadequate insulin secretion. At a dosage of 50 mg/kg, *Equisetum arvense* L. extract exhibited no discernible impact on hyperglycaemia or

IR, while administration at 200 mg/kg failed to elicit the anticipated therapeutic response. Conversely, administration at 100 mg/kg yielded significant reductions in blood glucose levels and enhancements in whole-body insulin sensitivity. There exists a well-established association between SIRT1 and glucose metabolism. Wang et al. reported that decreased SIRT1 levels can precipitate hepatic IR [285, 286], whereas SIRT1 activation can mitigate hepatic IR in the context of obesity [287]. Within adipocytes, SIRT1 promotes GLUT4 translocation and subsequent glucose uptake [285]. Additionally, SIRT1 is expressed in pancreatic ß-cells, where it augments glucose-stimulated insulin secretion [285, 288]. Studies indicate that selective upregulation of SIRT1 in ß-cells enhances glucose metabolism in mice [241], whereas its selective deletion leads to glucose intolerance [240]. Furthermore, data suggests that SIRT1 confers protection against ß-cell apoptosis [285, 289].

In our investigation, the *Equisetum arvense* L. extract induced an improved rate of body weight gain compared to the diabetic control group, particularly evident at doses of 100 mg/kg and 200 mg/kg. However, the body weight of rats treated with the extract did not reach that of the diabetic control rats by the end of the six-week experimental period. Additionally, our findings indicate a significant reduction in abdominal WAT weight in each of the STZ-treated groups compared to the healthy control. The reduction in abdominal WAT weight may be attributed to STZ therapy and the resultant relative insulin deficiency. Nonetheless, treatment with *Equisetum arvense* L. extract exhibited a moderate tendency to counteract this impact, likely attributable to the insulin-sensitizing properties of horsetail.

DCM, a prevalent and severe complication associated with both T1DM and T2DM, can manifest in the early or advanced stages of the disease, with prevention and treatment remaining unresolved [290]. Key features of DCM include left ventricular hypertrophy and progressive diastolic dysfunction, ultimately culminating in decompensated heart failure. Alongside well-established mechanisms, processes such as inflammation, metabolic disturbances, and oxidative stress may contribute to DCM pathogenesis [115, 291]. Despite ongoing research efforts, the molecular background of DCM remains incompletely understood. Various hypotheses propose mechanisms underlying DCM, encompassing ER stress, nitro-oxidative stress, mitochondrial dysfunction, autophagy, apoptosis, and alterations in specific structural and signalling proteins at post-translational and post-transcriptional levels [228, 252].

Reactive oxygen species (ROS) and advanced glycation end products (AGEs) produced as a result of oxidative stress are implicated in the disruption of structural and signalling protein conformation and function, leading to disturbances in myocyte calcium handling, as well as damage to the endoplasmic and sarcoplasmic reticulum. This damage is associated with reduced

activity of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a) pump [252, 290, 292, 293]. Moreover, oxidative stress can impact the activity of SIRT1, a key player in the pathogenesis of DCM, known for its beneficial effects on various signalling pathways. Specifically, SIRT1 has been shown to upregulate ERK, an anti-apoptotic MAP kinase [294], and deacetylate p53, thereby reducing apoptosis [295].

AGEs play a pivotal role in the pathogenesis of DCM by impairing key enzymes such as AMPK and SERCA2, while also contributing to the generation of reactive oxygen species and subsequent cellular damage. SIRT1 has been reported to possess the ability to activate or upregulate these enzymes, thereby potentially mitigating the effects of AGEs [290, 296]. Ongoing research efforts aim to develop advanced therapeutic strategies for the management of DCM, with the goal of achieving favourable outcomes while minimizing adverse effects and harmful drug interactions. Consequently, there is a growing scientific interest in traditional herbs and their bioactive compounds, which may serve as potential food supplements or as sources for the development of novel drug molecules [297-300].

We observed a significant increase in the heart weight index, indicative of cardiomegaly, in diabetic control rats and those treated with 200 mg/kg *Equisetum arvense* L. extract, compared to healthy control rats. However, animals treated with 50 mg/kg and 100 mg/kg of the extract did not show any difference in heart weight index compared to controls. These findings suggest that the STZ-induced diabetes model led to cardiomegaly, which was not effectively prevented by the 200 mg/kg dose of *Equisetum arvense* L. extract. Additionally, we noted a significant decrease in SIRT1 levels in diabetic control rats compared to healthy controls, and none of the administered doses of *Equisetum arvense* L. extract effectively increased SIRT1 levels. To the best of our knowledge, our research group was the first to investigate the potential SIRT1 activator effect of *Equisetum arvense* L. extract in an STZ-induced diabetic animal model.

It is important to note that the *Equisetum arvense* L. extract at a concentration of 100 mg/kg demonstrated a trend of elevating SIRT1 levels in cardiac muscle. While we acknowledge that the small sample size may limit the strength of our statistical analysis, it is noteworthy that a clear trend of SIRT1 elevation was observed at the dosage of *Equisetum arvense* L. extract that proved most effective in improving insulin sensitivity. We speculate that at this concentration, the Equisetum extract may ameliorate DCM symptoms through modulation of SIRT1, which plays a pivotal role in various molecular signalling pathways in cardiac muscle [193]. Elucidating the specific targets and mechanisms underlying this effect will necessitate further experimentation.

7 Summary

According to the WHO, obesity is positioned among the most widespread diseases in the whole world. The development of diabetes and CVD, as a result of uncontrollable weight gain, with repercussions on the increase in mortality, determined medical researches to find effective treatment in the prevention and treatment of obesity and implicitly its complications.

Our study aimed to delve into novel mechanisms of action and assess the therapeutic impact on insulin sensitivity in induced obesity and diabetes mellitus. We focused on two plant extracts renowned for their antidiabetic, adiposity-reducing, and cardioprotective properties: Fenugreek seeds (*Trigonella foenum-graecum* L.) and Horsetail extract *(Equisetum arvense* L.).

We used diet induced obese rats to investigate the effects of chronic oral treatment with fenugreek seeds and diosgenin on insulin sensitivity and weight gain. The obesity was induced by feeding the rats with HFD and 5% sucrose solution, for six weeks. For evaluate the effects on insulin sensitivity and weight gain, the rats were also treated with different doses of diosgenin or fenugreek seeds, 1, 10 and 50 mg/kg of Diosgenin or 0.6 g/kg Fenugreek seeds, mixed into the chow. After six weeks, we measured the following metabolic parameters: body weight, food and water intake, WAT weight, and insulin sensitivity.

Chronic administration of fenugreek resulted in increased body weight gain induced by a diet rich in fats and sugars. Additionally, abdominal adiposity and calorie intake were elevated in the fenugreek-treated group compared to both control and diet-induced obesity control animals. Despite its adverse effects on body weight, abdominal fat, and energy intake, fenugreek treatment did not adversely affect insulin sensitivity in peripheral tissues. Our results revealed that in high-calorie diet model, a lower dose of diosgenin, in conjunction with fenugreek, may heighten the risk of obesity. These findings warrant consideration, particularly for patients utilizing fenugreek as a dietary supplement to regulate blood glucose levels, either alone or combined with specific antidiabetic therapies. Although existing scientific reports suggest the fenugreek possesses insulin-sensitizing properties, our study failed to replicate this effect, possibly due to the relatively low dose utilized. We conclude that diosgenin in isolation does not lead to notable increases in body weight or fat accumulation. However, it likely interacts synergistically with other compounds present in fenugreek seeds. Further investigation is necessary to elucidate the mechanisms and roles of the active constituents involved.

In our continued investigation into the effects of bioactive compounds from plant extracts on metabolic parameters such as body weight gain, WAT, and insulin sensitivity, we observed promising outcomes with *Equisetum arvense* L. extract. Our study involved five groups of male

Wistar rats: a healthy control group, a diabetic control group, and three groups treated with varying doses of *Equisetum arvense* L. extract (50, 100, or 200 mg/kg) over a six-week period. Throughout the experiment, we assessed blood glucose levels, glucose tolerance, insulin sensitivity, SIRT1 levels, and other parameters relevant to diabetes and cardiomyopathy.

Our findings revealed that *Equisetum arvense* L. extract induced moderate beneficial changes in blood glucose levels and elevated SIRT1 levels in cardiomyocytes. Moreover, administration of the 100 mg/kg dose notably improved insulin sensitivity. Interestingly, the extract did not significantly affect body weight, adiposity, or heart weight index.

Based on our study findings, we conclude that *Equisetum arvense* L. extract shows promise as a supportive therapy, given its favourable impact on IR and blood glucose levels. Furthermore, its potential in averting diabetic cardiomyopathy could contribute to reduced morbidity in diabetes. However, further investigations are warranted to unravel the exact mechanisms of action of Equisetum extract and its effects across various organs.

8 References

1. Eidi, A., M. Eidi, and M. Sokhteh, *Effect of fenugreek (Trigonella foenum-graecum L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats.* Nutrition Research - NUTR RES, 2007. **27**: p. 728-733.
2. Ansari, R.M. and S.Y. Ansari. *Effectiveness of Fenugreek for Lowering Hemoglobin (HbA1c) in Patients with Self-Management of Type 2 Diabetes: A Randomized Controlled Trial*. 2011.
3. Kinlen, D., D. Cody, and D. O'Shea, *Complications of obesity.* Qjm, 2018. **111**(7): p. 437-443.
4. Novosad, S., et al., *Role of Obesity in Asthma Control, the Obesity-Asthma Phenotype.* Journal of allergy, 2013. **2013**: p. 538642.
5. Bhupathiraju, S.N. and F.B. Hu, *Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications.* Circ Res, 2016. **118**(11): p. 1723-35.
6. Garaulet, M., J.M. Ordovás, and J.A. Madrid, *The chronobiology, etiology and pathophysiology of obesity.* Int J Obes (Lond), 2010. **34**(12): p. 1667-83.
7. Stunkard, A.J. and T.A. Wadden, *Psychological aspects of severe obesity.* Am J Clin Nutr, 1992. **55**(2 Suppl): p. 524s-532s.
8. Pereira-Lancha, L.O., P.L. Campos-Ferraz, and A.H. Lancha, Jr., *Obesity: considerations about etiology, metabolism, and the use of experimental models.* Diabetes Metab Syndr Obes, 2012. **5**: p. 75-87.
9. Rohde, K., et al., *Genetics and epigenetics in obesity.* Metabolism, 2019. **92**: p. 37-50.
10. Chen, Y., et al., *Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity.* Endocrinology, 2002. **143**(7): p. 2469-77.
11. Makaronidis, J.M. and R.L. Batterham, *The role of gut hormones in the pathogenesis and management of obesity.* Current Opinion in Physiology, 2019. **12**: p. 1-11.
12. Nimptsch, K., S. Konigorski, and T. Pischon, *Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine.* Metabolism, 2019. **92**: p. 61-70.
13. Tilg, H. and A.R. Moschen, *Adipocytokines: mediators linking adipose tissue, inflammation and immunity.* Nat Rev Immunol, 2006. **6**(10): p. 772-83.
14. Chang, C.S., J.W. Ruan, and C.Y. Kao, *An overview of microbiome based strategies on anti-obesity.* Kaohsiung J Med Sci, 2019. **35**(1): p. 7-16.
15. Patel, S. and M. Saleem, *Major Complications and Management Options of Obesity.* Annals of International medical and Dental Research, 2017. **3**.
16. Herath Bandara, S.J. and C. Brown, *An analysis of adult obesity and hypertension in appalachia.* Glob J Health Sci, 2013. **5**(3): p. 127-38.
17. Kotsis, V., et al., *Impact of obesity on 24-hour ambulatory blood pressure and hypertension.* Hypertension, 2005. **45**(4): p. 602-7.
18. Pilitsi, E., et al., *Pharmacotherapy of obesity: Available medications and drugs under investigation.* Metabolism, 2019. **92**: p. 170-192.
19. Yannakoulia, M., et al., *Dietary modifications for weight loss and weight loss maintenance.* Metabolism, 2019. **92**: p. 153-162.
20. Muscogiuri, G., et al., *The management of very low-calorie ketogenic diet in obesity outpatient clinic: a practical guide.* Journal of Translational Medicine, 2019. **17**(1): p. 356.
21. Petridou, A., A. Siopi, and V. Mougios, *Exercise in the management of obesity.* Metabolism, 2019. **92**: p. 163-169.

22. Rena, G., D.G. Hardie, and E.R. Pearson, *The mechanisms of action of metformin.* Diabetologia, 2017. **60**(9): p. 1577-1585.
23. Wang, F.F., et al., *Pharmacologic therapy to induce weight loss in women who have obesity/overweight with polycystic ovary syndrome: a systematic review and network meta-analysis.* Obesity Reviews, 2018. **19**(10): p. 1424-1445.
24. Elmaraezy, A., et al., *Effect of metformin on maternal and neonatal outcomes in pregnant obese non-diabetic women: A meta-analysis.* Int J Reprod Biomed, 2017. **15**(8): p. 461-470.
25. Azegami, T., et al., *Nanogel-based nasal ghrelin vaccine prevents obesity.* Mucosal Immunol, 2017. **10**(5): p. 1351-1360.
26. Walters, W.A., Z. Xu, and R. Knight, *Meta-analyses of human gut microbes associated with obesity and IBD.* FEBS Lett, 2014. **588**(22): p. 4223-33.
27. Huo, L., J. Lyons, and D.J. Magliano, *Infectious and Environmental Influences on the Obesity Epidemic.* Curr Obes Rep, 2016. **5**(3): p. 375-82.
28. Cerdó, T., et al., *The Role of Probiotics and Prebiotics in the Prevention and Treatment of Obesity.* Nutrients, 2019. **11**(3).
29. Sams, V.G., et al., *Effect of bariatric surgery on systemic and adipose tissue inflammation.* Surg Endosc, 2016. **30**(8): p. 3499-504.
30. Kaaman, M., et al., *Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue.* Diabetologia, 2007. **50**(12): p. 2526-33.
31. Hansen, M., et al., *Adipose tissue mitochondrial respiration and lipolysis before and after a weight loss by diet and RYGB.* Obesity (Silver Spring), 2015. **23**(10): p. 2022-9.
32. Landgraf, R., et al., *Therapy of Type 2 Diabetes.* Exp Clin Endocrinol Diabetes, 2019. **127**(S 01): p. S73-s92.
33. Lin, X., et al., *Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025.* Sci Rep, 2020. **10**(1): p. 14790.
34. Magliano, D.J., E.J. Boyko, and I.D.F.D.A.t.e.s. committee, *IDF Diabetes Atlas*, in *Idf diabetes atlas*. 2021, International Diabetes Federation, 2021.: Brussels.
35. Durruty, P., M. Sanzana, and L. Sanhueza, *Pathogenesis of Type 2 Diabetes Mellitus*. 2019.
36. Mirtschink, P., et al., *Fructose metabolism, cardiometabolic risk, and the epidemic of coronary artery disease.* Eur Heart J, 2018. **39**(26): p. 2497-2505.
37. Mirmiran, P., et al., *Fatty acid quality and quantity of diet and risk of type 2 diabetes in adults: Tehran Lipid and Glucose Study.* J Diabetes Complications, 2018. **32**(7): p. 655-659.
38. Yerlikaya, A., et al., *Dietary and commercialized fructose: Sweet or sour?* Int Urol Nephrol, 2017. **49**(9): p. 1611-1620.
39. Zong, G., et al., *Gluten intake and risk of type 2 diabetes in three large prospective cohort studies of US men and women.* Diabetologia, 2018. **61**(10): p. 2164-2173.
40. Neel, B.A. and R.M. Sargis, *The paradox of progress: environmental disruption of metabolism and the diabetes epidemic.* Diabetes, 2011. **60**(7): p. 1838-48.
41. Reutrakul, S. and E. Van Cauter, *Interactions between sleep, circadian function, and glucose metabolism: implications for risk and severity of diabetes.* Ann N Y Acad Sci, 2014. **1311**: p. 151-73.
42. Mancuso, P., *The role of adipokines in chronic inflammation.* Immunotargets Ther, 2016. **5**: p. 47-56.
43. Unger, R.H. and L. Orci, *Glucagon and the A cell: physiology and pathophysiology (first two parts).* N Engl J Med, 1981. **304**(25): p. 1518-24.

44. Defronzo, R.A., *Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus.* Diabetes, 2009. **58**(4): p. 773-95.
45. Schwartz, S.S., et al., *The Time Is Right for a New Classification System for Diabetes: Rationale and Implications of the β-Cell-Centric Classification Schema.* Diabetes Care, 2016. **39**(2): p. 179-86.
46. *Medical Complications of Type 2 Diabetes*. 2011, IntechOpen: Rijeka.
47. Martín-Peláez, S., M. Fito, and O. Castaner, *Mediterranean Diet Effects on Type 2 Diabetes Prevention, Disease Progression, and Related Mechanisms. A Review.* Nutrients, 2020. **12**(8).
48. Michalczyk, M.M., et al., *The Effects of a Low Calorie Ketogenic Diet on Glycaemic Control Variables in Hyperinsulinemic Overweight/Obese Females.* Nutrients, 2020. **12**(6).
49. Yang, D., et al., *Physical Exercise as Therapy for Type 2 Diabetes Mellitus: From Mechanism to Orientation.* Ann Nutr Metab, 2019. **74**(4): p. 313-321.
50. Kirwan, J.P., J. Sacks, and S. Nieuwoudt, *The essential role of exercise in the management of type 2 diabetes.* Cleve Clin J Med, 2017. **84**(7 Suppl 1): p. S15-S21.
51. Rodríguez-Saldaña, J., *The Diabetes Textbook Clinical Principles, Patient Management and Public Health Issues: Clinical Principles, Patient Management and Public Health Issues*. 2019.
52. ElSayed, N.A., et al., *9. Pharmacologic Approaches to Glycemic Treatment: Standards of Care in Diabetes-2023.* Diabetes Care, 2023. **46**(Suppl 1): p. S140-s157.
53. Garber, A.J., et al., *Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Comprehensive Type 2 Diabetes Management Algorithm - 2018 Executive Summary.* Endocr Pract, 2018. **24**(1): p. 91-120.
54. Excellence, N.I.o.H.a.C. *Type 2 diabetes in adults: management*. 2015 29/06/2022 [cited 2024; Available from: https://www.nice.org.uk/guidance/ng28.
55. Rendell, M., *The role of sulphonylureas in the management of type 2 diabetes mellitus.* Drugs, 2004. **64**(12): p. 1339-58.
56. Inzucchi, S.E., et al., *Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes.* Diabetes Care, 2015. **38**(1): p. 140-9.
57. Desai, C., *Meyler's side effects of drugs: The international encyclopedia of adverse drug reactions and interactions*, in *Indian J Pharmacol*. 2016, Copyright: © Indian Journal of Pharmacology. p. 224.
58. Corcoran, C. and T.F. Jacobs, *Metformin*, in *StatPearls*. 2024, StatPearls Publishing Copyright © 2024, StatPearls Publishing LLC.: Treasure Island (FL).
59. *Standards of medical care in diabetes--2012.* Diabetes Care, 2012. **35 Suppl 1**(Suppl 1): p. S11-63.
60. Derosa, G. and P. Maffioli, *α-Glucosidase inhibitors and their use in clinical practice.* Arch Med Sci, 2012. **8**(5): p. 899-906.
61. Nissen, S.E. and K. Wolski, *Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality.* Arch Intern Med, 2010. **170**(14): p. 1191-1201.
62. Kahn, S.E., et al., *Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy.* N Engl J Med, 2006. **355**(23): p. 2427-43.

63. Home, P.D., et al., *Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial.* Lancet, 2009. **373**(9681): p. 2125-35.
64. Drucker, D.J., *Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1.* Cell Metab, 2018. **27**(4): p. 740-756.
65. Nauck, M.A., et al., *GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art.* Mol Metab, 2021. **46**: p. 101102.
66. Vella, A., *Mechanism of action of DPP-4 inhibitors--new insights.* J Clin Endocrinol Metab, 2012. **97**(8): p. 2626-8.
67. Scheen, A.J., *A review of gliptins in 2011.* Expert Opin Pharmacother, 2012. **13**(1): p. 81-99.
68. Barzilai, N., et al., *Efficacy and tolerability of sitagliptin monotherapy in elderly patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial.* Current Medical Research and Opinion, 2011. **27**(5): p. 1049-1058.
69. Davies, M.J., et al., *Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD).* Diabetes Care, 2018. **41**(12): p. 2669-2701.
70. Buse, J.B., et al., *2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD).* Diabetes Care, 2020. **43**(2): p. 487-493.
71. *Introduction: Standards of Medical Care in Diabetes-2022.* Diabetes Care, 2022. **45**(Suppl 1): p. S1-s2.
72. Garber, A.J., et al., *Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Comprehensive Type 2 Diabetes Management Algorithm - 2020 Executive Summary.* Endocr Pract, 2020. **26**(1): p. 107-139.
73. Huang, J., et al., *Adverse event profiles of dipeptidyl peptidase-4 inhibitors: data mining of the public version of the FDA adverse event reporting system.* BMC Pharmacology and Toxicology, 2020. **21**(1): p. 68.
74. Pathak, R. and M.B. Bridgeman, *Dipeptidyl Peptidase-4 (DPP-4) Inhibitors In the Management of Diabetes.* P t, 2010. **35**(9): p. 509-13.
75. DeFronzo, R.A., L. Norton, and M. Abdul-Ghani, *Renal, metabolic and cardiovascular considerations of SGLT2 inhibition.* Nat Rev Nephrol, 2017. **13**(1): p. 11-26.
76. Skrtić, M., et al., *Characterisation of glomerular haemodynamic responses to SGLT2 inhibition in patients with type 1 diabetes and renal hyperfiltration.* Diabetologia, 2014. **57**(12): p. 2599-602.
77. Takagi, S., et al., *Ipragliflozin improves mitochondrial abnormalities in renal tubules induced by a high-fat diet.* J Diabetes Investig, 2018. **9**(5): p. 1025-1032.
78. Fonseca-Correa, J.I. and R. Correa-Rotter, *Sodium-Glucose Cotransporter 2 Inhibitors Mechanisms of Action: A Review.* Front Med (Lausanne), 2021. **8**: p. 777861.
79. Neal, B., et al., *Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes.* N Engl J Med, 2017. **377**(7): p. 644-657.
80. Ghanim, H., et al., *Dapagliflozin Suppresses Hepcidin And Increases Erythropoiesis.* J Clin Endocrinol Metab, 2020. **105**(4).
81. Lee, T.M., N.C. Chang, and S.Z. Lin, *Dapagliflozin, a selective SGLT2 Inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization via STAT3 signaling in infarcted rat hearts.* Free Radic Biol Med, 2017. **104**: p. 298-310.

82. Bonnet, F. and A.J. Scheen, *Effects of SGLT2 inhibitors on systemic and tissue low-grade inflammation: The potential contribution to diabetes complications and cardiovascular disease.* Diabetes Metab, 2018. **44**(6): p. 457-464.
83. Cassis, P., et al., *SGLT2 inhibitor dapagliflozin limits podocyte damage in proteinuric nondiabetic nephropathy*. JCI Insight, 2018. **3**(15).
84. *9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2022.* Diabetes Care, 2022. **45**(Suppl 1): p. S125-s143.
85. Zelniker, T.A., et al., *SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials.* Lancet, 2019. **393**(10166): p. 31-39.
86. *KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease.* Kidney Int, 2022. **102**(5s): p. S1-s127.
87. Pereira, M.J. and J.W. Eriksson, *Emerging Role of SGLT-2 Inhibitors for the Treatment of Obesity.* Drugs, 2019. **79**(3): p. 219-230.
88. Cusi, K., et al., *American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD).* Endocr Pract, 2022. **28**(5): p. 528-562.
89. Halimi, S. and B. Vergès, *Adverse effects and safety of SGLT-2 inhibitors.* Diabetes Metab, 2014. **40**(6 Suppl 1): p. S28-34.
90. McGill, J.B. and S. Subramanian, *Safety of Sodium-Glucose Co-Transporter 2 Inhibitors.* Am J Cardiol, 2019. **124 Suppl 1**: p. S45-s52.
91. Kahn, C.R., *The molecular mechanism of insulin action.* Annu Rev Med, 1985. **36**: p. 429-51.
92. Posner, B.I., *Insulin Signalling: The Inside Story.* Can J Diabetes, 2017. **41**(1): p. 108-113.
93. Jaldin-Fincati, J.R., et al., *Update on GLUT4 Vesicle Traffic: A Cornerstone of Insulin Action.* Trends Endocrinol Metab, 2017. **28**(8): p. 597-611.
94. Heise, T., et al., *Faster-acting insulin aspart: earlier onset of appearance and greater early pharmacokinetic and pharmacodynamic effects than insulin aspart.* Diabetes Obes Metab, 2015. **17**(7): p. 682-8.
95. Wong, E.Y. and L. Kroon, *Ultra-Rapid-Acting Insulins: How Fast Is Really Needed?* Clin Diabetes, 2021. **39**(4): p. 415-423.
96. Pilar, D., S. María, and S. Lilian, *Pathogenesis of Type 2 Diabetes Mellitus*, in *Type 2 Diabetes*, S. Mira, Editor. 2019, IntechOpen: Rijeka. p. Ch. 2.
97. Rinninella, E., et al., *What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases.* Microorganisms, 2019. **7**(1).
98. Slouha, E., et al., *Type-2 Diabetes Mellitus and the Gut Microbiota: Systematic Review.* Cureus, 2023. **15**(11): p. e49740.
99. Joseph, B. and D. Jini, *Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency.* Asian Pac J Trop Dis, 2013. **3**(2): p. 93-102.
100. Zheng, Y., et al., *Interactions Between Gut Microbiota, Host, and Herbal Medicines: A Review of New Insights Into the Pathogenesis and Treatment of Type 2 Diabetes.* Front Cell Infect Microbiol, 2020. **10**: p. 360.
101. Saeed, M., et al., *Nutritional analysis and antioxidant activity of bitter gourd (Momordica charantia) from Pakistan.* Pharmacologyonline, 2010. **1**: p. 252-260.
102. Mahmoud, M.F., et al., *Quercetin protects against diabetes-induced exaggerated vasoconstriction in rats: effect on low grade inflammation.* PLoS One, 2013. **8**(5): p. e63784.

103. Lal, S., *A Review: Medicinal Plants and its Impact on Diabetes.* World Journal of Pharmaceutical Research, 2012.
104. Richter, E., et al., *The Effects of Momordica charantia on Type 2 Diabetes Mellitus and Alzheimer's Disease.* Int J Mol Sci, 2023. **24**(5).
105. Bortolotti, M., D. Mercatelli, and L. Polito, *Momordica charantia, a Nutraceutical Approach for Inflammatory Related Diseases.* Front Pharmacol, 2019. **10**: p. 486.
106. Dillmann, W.H., *Diabetic Cardiomyopathy.* Circ Res, 2019. **124**(8): p. 1160-1162.
107. Rubler, S., et al., *New type of cardiomyopathy associated with diabetic glomerulosclerosis.* Am J Cardiol, 1972. **30**(6): p. 595-602.
108. Elliott, P., et al., *Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases.* Eur Heart J, 2008. **29**(2): p. 270-6.
109. Jia, G., A. Whaley-Connell, and J.R. Sowers, *Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease.* Diabetologia, 2018. **61**(1): p. 21-28.
110. Seferović, P.M. and W.J. Paulus, *Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes.* Eur Heart J, 2015. **36**(27): p. 1718-27, 1727a-1727c.
111. Schocken, D.D., et al., *Prevention of heart failure: a scientific statement from the American Heart Association Councils on Epidemiology and Prevention, Clinical Cardiology, Cardiovascular Nursing, and High Blood Pressure Research; Quality of Care and Outcomes Research Interdisciplinary Working Group; and Functional Genomics and Translational Biology Interdisciplinary Working Group.* Circulation, 2008. **117**(19): p. 2544-65.
112. Gulsin, G.S., L. Athithan, and G.P. McCann, *Diabetic cardiomyopathy: prevalence, determinants and potential treatments.* Ther Adv Endocrinol Metab, 2019. **10**: p. 2042018819834869.
113. Kannel, W.B., M. Hjortland, and W.P. Castelli, *Role of diabetes in congestive heart failure: the Framingham study.* Am J Cardiol, 1974. **34**(1): p. 29-34.
114. Aronow, W.S. and C. Ahn, *Incidence of heart failure in 2,737 older persons with and without diabetes mellitus.* Chest, 1999. **115**(3): p. 867-8.
115. Jia, G., M.A. Hill, and J.R. Sowers, *Diabetic Cardiomyopathy: An Update of Mechanisms Contributing to This Clinical Entity.* Circ Res, 2018. **122**(4): p. 624-638.
116. Boonman-de Winter, L.J., et al., *High prevalence of previously unknown heart failure and left ventricular dysfunction in patients with type 2 diabetes.* Diabetologia, 2012. **55**(8): p. 2154-62.
117. Stratton, I.M., et al., *Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study.* Bmj, 2000. **321**(7258): p. 405-12.
118. Toedebusch, R., A. Belenchia, and L. Pulakat, *Diabetic Cardiomyopathy: Impact of Biological Sex on Disease Development and Molecular Signatures.* Front Physiol, 2018. **9**: p. 453.
119. Borghetti, G., et al., *Diabetic Cardiomyopathy: Current and Future Therapies. Beyond Glycemic Control.* Front Physiol, 2018. **9**: p. 1514.
120. Dandamudi, S., et al., *The prevalence of diabetic cardiomyopathy: a population-based study in Olmsted County, Minnesota.* J Card Fail, 2014. **20**(5): p. 304-9.
121. Fang, Z.Y., J.B. Prins, and T.H. Marwick, *Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications.* Endocr Rev, 2004. **25**(4): p. 543-67.
122. Pappachan, J.M., et al., *Diabetic cardiomyopathy: Pathophysiology, diagnostic evaluation and management.* World J Diabetes, 2013. **4**(5): p. 177-89.

123. Jia, G., V.G. DeMarco, and J.R. Sowers, *Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy.* Nat Rev Endocrinol, 2016. **12**(3): p. 144-53.
124. Lazo, M., et al., *Soluble receptor for advanced glycation end products and the risk for incident heart failure: The Atherosclerosis Risk in Communities Study.* Am Heart J, 2015. **170**(5): p. 961-7.
125. Xie, J., et al., *Cellular signalling of the receptor for advanced glycation end products (RAGE).* Cell Signal, 2013. **25**(11): p. 2185-97.
126. Zhang, M., et al., *Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of Nox2 (gp91phox)-containing NADPH oxidase.* Circulation, 2006. **113**(9): p. 1235-43.
127. Fukami, K., S. Yamagishi, and S. Okuda, *Role of AGEs-RAGE system in cardiovascular disease.* Curr Pharm Des, 2014. **20**(14): p. 2395-402.
128. Hu, P., et al., *ERK and Akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells.* Int J Mol Med, 2012. **29**(4): p. 613-8.
129. Travers, J.G., et al., *Cardiac Fibrosis: The Fibroblast Awakens.* Circ Res, 2016. **118**(6): p. 1021-40.
130. Muñoz-Córdova, F., et al., *Novel Insights Into the Pathogenesis of Diabetic Cardiomyopathy and Pharmacological Strategies.* Front Cardiovasc Med, 2021. **8**: p. 707336.
131. Ramesh, P., et al., *Role of inflammation in diabetic cardiomyopathy.* Ther Adv Endocrinol Metab, 2022. **13**: p. 20420188221083530.
132. Xu, D., et al., *Inhibition of miR-223 attenuates the NLRP3 inflammasome activation, fibrosis, and apoptosis in diabetic cardiomyopathy.* Life Sci, 2020. **256**: p. 117980.
133. Theofilis, P., et al., *Diabetes Mellitus and Heart Failure: Epidemiology, Pathophysiologic Mechanisms, and the Role of SGLT2 Inhibitors.* Life (Basel), 2023. **13**(2).
134. Zhang, D., et al., *Hyperglycemia-induced secretion of endothelial heparanase stimulates a vascular endothelial growth factor autocrine network in cardiomyocytes that promotes recruitment of lipoprotein lipase.* Arterioscler Thromb Vasc Biol, 2013. **33**(12): p. 2830-8.
135. Schulze, P.C., K. Drosatos, and I.J. Goldberg, *Lipid Use and Misuse by the Heart.* Circ Res, 2016. **118**(11): p. 1736-51.
136. Salvatore, T., et al., *The Diabetic Cardiomyopathy: The Contributing Pathophysiological Mechanisms.* Front Med (Lausanne), 2021. **8**: p. 695792.
137. van de Weijer, T., V.B. Schrauwen-Hinderling, and P. Schrauwen, *Lipotoxicity in type 2 diabetic cardiomyopathy.* Cardiovasc Res, 2011. **92**(1): p. 10-8.
138. Tang, Z., et al., *Oxidative Stress Signaling Mediated Pathogenesis of Diabetic Cardiomyopathy.* Oxid Med Cell Longev, 2022. **2022**: p. 5913374.
139. Nakamura, K., et al., *Pathophysiology and Treatment of Diabetic Cardiomyopathy and Heart Failure in Patients with Diabetes Mellitus.* Int J Mol Sci, 2022. **23**(7).
140. Hill, M.F., *Emerging role for antioxidant therapy in protection against diabetic cardiac complications: experimental and clinical evidence for utilization of classic and new antioxidants.* Curr Cardiol Rev, 2008. **4**(4): p. 259-68.
141. Huo, J.L., et al., *Diabetic cardiomyopathy: Early diagnostic biomarkers, pathogenetic mechanisms, and therapeutic interventions.* Cell Death Discov, 2023. **9**(1): p. 256.
142. Gilbert, R.E. and H. Krum, *Heart failure in diabetes: effects of anti-hyperglycaemic drug therapy.* Lancet, 2015. **385**(9982): p. 2107-17.
143. Seino, S., *Cell signalling in insulin secretion: the molecular targets of ATP, cAMP and sulfonylurea.* Diabetologia, 2012. **55**(8): p. 2096-108.

144. Tzoulaki, I., et al., *Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database.* BMJ, 2009. **339**: p. b4731.
145. Yang, F., et al., *Metformin Inhibits the NLRP3 Inflammasome via AMPK/mTOR-dependent Effects in Diabetic Cardiomyopathy.* Int J Biol Sci, 2019. **15**(5): p. 1010-1019.
146. Marfella, R., et al., *Sodium/glucose cotransporter 2 (SGLT2) inhibitors improve cardiac function by reducing JunD expression in human diabetic hearts.* Metabolism, 2022. **127**: p. 154936.
147. Tomasoni, D., et al., *Sodium-glucose co-transporter 2 inhibitors as an early, first-line therapy in patients with heart failure and reduced ejection fraction.* Eur J Heart Fail, 2022. **24**(3): p. 431-441.
148. Arow, M., et al., *Sodium-glucose cotransporter 2 inhibitor Dapagliflozin attenuates diabetic cardiomyopathy.* Cardiovasc Diabetol, 2020. **19**(1): p. 7.
149. Madonna, R., et al., *Empagliflozin inhibits excessive autophagy through the AMPK/GSK3β signalling pathway in diabetic cardiomyopathy.* Cardiovasc Res, 2023. **119**(5): p. 1175-1189.
150. Kadosaka, T., et al., *Empagliflozin attenuates arrhythmogenesis in diabetic cardiomyopathy by normalizing intracellular Ca(2+) handling in ventricular cardiomyocytes.* Am J Physiol Heart Circ Physiol, 2023. **324**(3): p. H341-h354.
151. Xing, Y.J., et al., *A SGLT2 Inhibitor Dapagliflozin Alleviates Diabetic Cardiomyopathy by Suppressing High Glucose-Induced Oxidative Stress in vivo and in vitro.* Front Pharmacol, 2021. **12**: p. 708177.
152. Brown, A.J.M., et al., *A randomized controlled trial of dapagliflozin on left ventricular hypertrophy in people with type two diabetes: the DAPA-LVH trial.* Eur Heart J, 2020. **41**(36): p. 3421-3432.
153. Wang, D., et al., *Glucagon-like peptide-1 protects against cardiac microvascular injury in diabetes via a cAMP/PKA/Rho-dependent mechanism.* Diabetes, 2013. **62**(5): p. 1697-708.
154. Wu, L., et al., *Glucagon-like peptide-1 ameliorates cardiac lipotoxicity in diabetic cardiomyopathy via the PPARα pathway.* Aging Cell, 2018. **17**(4): p. e12763.
155. Almutairi, M., et al., *The GLP-1 Receptor Agonist Liraglutide Increases Myocardial Glucose Oxidation Rates via Indirect Mechanisms and Mitigates Experimental Diabetic Cardiomyopathy.* Can J Cardiol, 2021. **37**(1): p. 140-150.
156. Seufert, J. and B. Gallwitz, *The extra-pancreatic effects of GLP-1 receptor agonists: a focus on the cardiovascular, gastrointestinal and central nervous systems.* Diabetes Obes Metab, 2014. **16**(8): p. 673-88.
157. Qian, P., et al., *A novel oral glucagon-like peptide 1 receptor agonist protects against diabetic cardiomyopathy via alleviating cardiac lipotoxicity induced mitochondria dysfunction.* Biochem Pharmacol, 2020. **182**: p. 114209.
158. Mulvihill, E.E. and D.J. Drucker, *Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors.* Endocr Rev, 2014. **35**(6): p. 992-1019.
159. Oe, H., et al., *Comparison of effects of sitagliptin and voglibose on left ventricular diastolic dysfunction in patients with type 2 diabetes: results of the 3D trial.* Cardiovasc Diabetol, 2015. **14**: p. 83.
160. Yamanouchi, T., *Concomitant therapy with pioglitazone and insulin for the treatment of type 2 diabetes.* Vasc Health Risk Manag, 2010. **6**: p. 189-97.
161. Vieira, R., et al., *Sugar-Lowering Drugs for Type 2 Diabetes Mellitus and Metabolic Syndrome-Review of Classical and New Compounds: Part-I.* Pharmaceuticals (Basel), 2019. **12**(4).

162. Murarka, S. and M.R. Movahed, *Diabetic cardiomyopathy.* J Card Fail, 2010. **16**(12): p. 971-9.
163. Al-Rasheed, N.M., et al., *Simvastatin Ameliorates Diabetic Cardiomyopathy by Attenuating Oxidative Stress and Inflammation in Rats.* Oxid Med Cell Longev, 2017. **2017**: p. 1092015.
164. Min, J.J., et al., *Effects of Pravastatin on Type 1 Diabetic Rat Heart with or without Blood Glycemic Control.* J Diabetes Res, 2018. **2018**: p. 1067853.
165. Kanda, M., K. Satoh, and K. Ichihara, *Effects of atorvastatin and pravastatin on glucose tolerance in diabetic rats mildly induced by streptozotocin.* Biol Pharm Bull, 2003. **26**(12): p. 1681-4.
166. Koh, K.K., et al., *Differential metabolic effects of pravastatin and simvastatin in hypercholesterolemic patients.* Atherosclerosis, 2009. **204**(2): p. 483-90.
167. Herman-Edelstein, M., et al., *Expression of the SARS-CoV-2 receptorACE2 in human heart is associated with uncontrolled diabetes, obesity, and activation of the renin angiotensin system.* Cardiovasc Diabetol, 2021. **20**(1): p. 90.
168. Bernardi, S., et al., *Update on RAAS Modulation for the Treatment of Diabetic Cardiovascular Disease.* J Diabetes Res, 2016. **2016**: p. 8917578.
169. Azizi, M. and J. Ménard, *Renin inhibitors and cardiovascular and renal protection: an endless quest?* Cardiovasc Drugs Ther, 2013. **27**(2): p. 145-53.
170. Liu, W., et al., *Spironolactone Protects against Diabetic Cardiomyopathy in Streptozotocin-Induced Diabetic Rats.* J Diabetes Res, 2018. **2018**: p. 9232065.
171. Verdonschot, J.A.J., et al., *Proteomic mechanistic profile of patients with diabetes at risk of developing heart failure: insights from the HOMAGE trial.* Cardiovasc Diabetol, 2021. **20**(1): p. 163.
172. Sharma, V., et al., *Metoprolol improves cardiac function and modulates cardiac metabolism in the streptozotocin-diabetic rat.* Am J Physiol Heart Circ Physiol, 2008. **294**(4): p. H1609-20.
173. Amour, J., et al., *Preservation of the positive lusitropic effect of beta-adrenoceptors stimulation in diabetic cardiomyopathy.* Anesth Analg, 2008. **107**(4): p. 1130-8.
174. Adeghate, E., et al., *Medicinal chemistry of drugs used in diabetic cardiomyopathy.* Curr Med Chem, 2010. **17**(6): p. 517-51.
175. Tang, S.G., et al., *Trimetazidine prevents diabetic cardiomyopathy by inhibiting Nox2/TRPC3-induced oxidative stress.* J Pharmacol Sci, 2019. **139**(4): p. 311-318.
176. Zhang, L., et al., *Early administration of trimetazidine attenuates diabetic cardiomyopathy in rats by alleviating fibrosis, reducing apoptosis and enhancing autophagy.* J Transl Med, 2016. **14**(1): p. 109.
177. Li, Y.J., et al., *Improvement of mechanical heart function by trimetazidine in db/db mice.* Acta Pharmacol Sin, 2010. **31**(5): p. 560-9.
178. Dugbartey, G.J., et al., *Alpha-lipoic acid treatment improves adverse cardiac remodelling in the diabetic heart - The role of cardiac hydrogen sulfide-synthesizing enzymes.* Biochem Pharmacol, 2022. **203**: p. 115179.
179. Li, C.J., et al., *Cardiac fibrosis and dysfunction in experimental diabetic cardiomyopathy are ameliorated by alpha-lipoic acid.* Cardiovasc Diabetol, 2012. **11**: p. 73.
180. Giannetta, E., et al., *Chronic Inhibition of cGMP phosphodiesterase 5A improves diabetic cardiomyopathy: a randomized, controlled clinical trial using magnetic resonance imaging with myocardial tagging.* Circulation, 2012. **125**(19): p. 2323-33.
181. Pofi, R., et al., *Sex-specific effects of daily tadalafil on diabetic heart kinetics in RECOGITO, a randomized, double-blind, placebo-controlled trial.* Sci Transl Med, 2022. **14**(649): p. eabl8503.

182. Nickel, A., J. Löffler, and C. Maack, *Myocardial energetics in heart failure.* Basic Res Cardiol, 2013. **108**(4): p. 358.
183. Senanayake, E.L., et al., *Multicentre double-blind randomized controlled trial of perhexiline as a metabolic modulator to augment myocardial protection in patients with left ventricular hypertrophy undergoing cardiac surgery.* Eur J Cardiothorac Surg, 2015. **48**(3): p. 354-62.
184. Velmurugan, G.V., et al., *Defective Nrf2-dependent redox signalling contributes to microvascular dysfunction in type 2 diabetes.* Cardiovasc Res, 2013. **100**(1): p. 143-50.
185. Hsuan, C.F., et al., *Emerging Therapy for Diabetic Cardiomyopathy: From Molecular Mechanism to Clinical Practice.* Biomedicines, 2023. **11**(3).
186. Huynh, K., et al., *Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways.* Pharmacol Ther, 2014. **142**(3): p. 375-415.
187. Abdullah, C.S. and Z.Q. Jin, *Targeted deletion of T-cell S1P receptor 1 ameliorates cardiac fibrosis in streptozotocin-induced diabetic mice.* Faseb j, 2018. **32**(10): p. 5426-5435.
188. Luo, B., et al., *NLRP3 gene silencing ameliorates diabetic cardiomyopathy in a type 2 diabetes rat model.* PLoS One, 2014. **9**(8): p. e104771.
189. Luo, B., et al., *Rosuvastatin alleviates diabetic cardiomyopathy by inhibiting NLRP3 inflammasome and MAPK pathways in a type 2 diabetes rat model.* Cardiovasc Drugs Ther, 2014. **28**(1): p. 33-43.
190. Chen, H., et al., *Micro-RNA-195 and -451 regulate the LKB1/AMPK signaling axis by targeting MO25.* PLoS One, 2012. **7**(7): p. e41574.
191. Cheng, Y., et al., *Transplantation of bone marrow-derived endothelial progenitor cells attenuates myocardial interstitial fibrosis and cardiac dysfunction in streptozotocin-induced diabetic rats.* Int J Mol Med, 2012. **30**(4): p. 870-6.
192. Meloni, M., et al., *Nerve growth factor gene therapy using adeno-associated viral vectors prevents cardiomyopathy in type 1 diabetic mice.* Diabetes, 2012. **61**(1): p. 229-40.
193. Karbasforooshan, H. and G. Karimi, *The role of SIRT1 in diabetic cardiomyopathy.* Biomed Pharmacother, 2017. **90**: p. 386-392.
194. Li, K., et al., *Tetrahydrocurcumin Ameliorates Diabetic Cardiomyopathy by Attenuating High Glucose-Induced Oxidative Stress and Fibrosis via Activating the SIRT1 Pathway.* Oxid Med Cell Longev, 2019. **2019**: p. 6746907.
195. Zhai, M., et al., *Melatonin ameliorates myocardial ischemia reperfusion injury through SIRT3-dependent regulation of oxidative stress and apoptosis.* J Pineal Res, 2017. **63**(2).
196. Wu, X., et al., *Autophagy and cardiac diseases: Therapeutic potential of natural products.* Med Res Rev, 2021. **41**(1): p. 314-341.
197. Gao, Y., et al., *The Beneficial Effects of Chinese Herbal Monomers on Ameliorating Diabetic Cardiomyopathy via Nrf2 Signaling.* Oxid Med Cell Longev, 2022. **2022**: p. 3959390.
198. Pradeep, S.R. and K. Srinivasan, *Alleviation of Cardiac Damage by Dietary Fenugreek (Trigonella foenum-graecum) Seeds is Potentiated by Onion (Allium cepa) in Experimental Diabetic Rats via Blocking Renin-Angiotensin System.* Cardiovasc Toxicol, 2018. **18**(3): p. 221-231.
199. Bafadam, S., et al., *Cardioprotective effects of Fenugreek (Trigonella foenum-graceum) seed extract in streptozotocin induced diabetic rats.* J Cardiovasc Thorac Res, 2021. **13**(1): p. 28-36.
200. Goyal, S., N. Gupta, and S. Chatterjee, *Investigating Therapeutic Potential of Trigonella foenum-graecum L. as Our Defense Mechanism against Several Human Diseases.* J Toxicol, 2016. **2016**: p. 1250387.

201. Ahmad, A., et al., *Fenugreek a multipurpose crop: Potentialities and improvements.* Saudi J Biol Sci, 2016. **23**(2): p. 300-10.
202. Syed, Q.A., et al., *Nutritional and therapeutic properties of fenugreek (Trigonella foenum-graecum): a review.* International Journal of Food Properties, 2020. **23:1**, 1777-1791.
203. Visuvanathan, T., et al., *Revisiting Trigonella foenum-graecum L.: Pharmacology and Therapeutic Potentialities.* Plants (Basel), 2022. **11**(11).
204. Broca, C., et al., *4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin secretion.* Eur J Pharmacol, 2000. **390**(3): p. 339-45.
205. Rababah, T.M., et al., *Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants.* Nat Prod Res, 2011. **25**(6): p. 596-605.
206. Nagulapalli Venkata, K.C., et al., *A small plant with big benefits: Fenugreek (Trigonella foenum-graecum Linn.) for disease prevention and health promotion.* Mol Nutr Food Res, 2017. **61**(6).
207. Neelakantan, N., et al., *Effect of fenugreek (Trigonella foenum-graecum L.) intake on glycemia: a meta-analysis of clinical trials.* Nutr J, 2014. **13**: p. 7.
208. Fuller, S. and J.M. Stephens, *Diosgenin, 4-hydroxyisoleucine, and fiber from fenugreek: mechanisms of actions and potential effects on metabolic syndrome.* Adv Nutr, 2015. **6**(2): p. 189-97.
209. Kiss, R., et al., *Diosgenin and Its Fenugreek Based Biological Matrix Affect Insulin Resistance and Anabolic Hormones in a Rat Based Insulin Resistance Model.* Biomed Res Int, 2019. **2019**: p. 7213913.
210. Tharaheswari, M., et al., *Trigonelline and diosgenin attenuate ER stress, oxidative stress-mediated damage in pancreas and enhance adipose tissue PPARγ activity in type 2 diabetic rats.* Mol Cell Biochem, 2014. **396**(1-2): p. 161-74.
211. Jesus, M., et al., *Diosgenin: Recent Highlights on Pharmacology and Analytical Methodology.* J Anal Methods Chem, 2016. **2016**: p. 4156293.
212. Uemura, T., et al., *Diosgenin present in fenugreek improves glucose metabolism by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues.* Mol Nutr Food Res, 2010. **54**(11): p. 1596-608.
213. Pallag, A., et al., *Equisetum arvense L. Extract Induces Antibacterial Activity and Modulates Oxidative Stress, Inflammation, and Apoptosis in Endothelial Vascular Cells Exposed to Hyperosmotic Stress.* Oxid Med Cell Longev, 2018. **2018**: p. 3060525.
214. Dragos, D., et al., *Phytomedicine in Joint Disorders.* Nutrients, 2017. **9**(1).
215. Asgharikhatooni, A., et al., *The effect of equisetum arvense (horse tail) ointment on wound healing and pain intensity after episiotomy: a randomized placebo-controlled trial.* Iran Red Crescent Med J, 2015. **17**(3): p. e25637.
216. Gründemann, C., et al., *Equisetum arvense (common horsetail) modulates the function of inflammatory immunocompetent cells.* BMC Complement Altern Med, 2014. **14**: p. 283.
217. Al-Snafi, A., *The pharmacology of Equisetum arvense-A review.* IOSR Journal of Pharmacy 2017; 7(2): 31-42, 2017. **7**: p. 31-42.
218. Mimica-Dukic, N., et al., *Phenolic compounds in field horsetail (Equisetum arvense L.) as natural antioxidants.* Molecules, 2008. **13**(7): p. 1455-64.
219. Carneiro, D.M., et al., *Randomized, Double-Blind Clinical Trial to Assess the Acute Diuretic Effect of Equisetum arvense (Field Horsetail) in Healthy Volunteers.* Evid Based Complement Alternat Med, 2014. **2014**: p. 760683.
220. Asgarpanah, J. and E. Roohi, *Phytochemistry and pharmacological properties of Equisetum arvense L.* Journal of Medicinal Plants Research, 2012. **6**.

221. Foss, K., K.E. Przybyłowicz, and T. Sawicki, *Antioxidant Activity and Profile of Phenolic Compounds in Selected Herbal Plants.* Plant Foods Hum Nutr, 2022. **77**(3): p. 383-389.
222. Abdullah, R., et al., *Nephroprotective effects of Equisetum ramosissimum L. extract in streptozotocin-induced diabetic rats.* Pharmacia, 2024. **71**: p. 1-11.
223. Safiyeh, S., et al., *Antidiabetic effect of Equisetum arvense L. (Equisetaceae) in streptozotocin-induced diabetes in male rats.* Pak J Biol Sci, 2007. **10**(10): p. 1661-6.
224. Soleimani, S., F.F. Azarbaizani, and V. Nejati, *The effect of Equisetum arvense L. (Equisetaceae) in histological changes of pancreatic beta-cells in streptozotocin-induced diabetic in rats.* Pak J Biol Sci, 2007. **10**(23): p. 4236-40.
225. Iside, C., et al., *SIRT1 Activation by Natural Phytochemicals: An Overview.* Front Pharmacol, 2020. **11**: p. 1225.
226. Zhang, W., et al., *Sirt1 Inhibits Oxidative Stress in Vascular Endothelial Cells.* Oxid Med Cell Longev, 2017. **2017**: p. 7543973.
227. García-Barrado, M.J., et al., *Role of Flavonoids in The Interactions among Obesity, Inflammation, and Autophagy.* Pharmaceuticals (Basel), 2020. **13**(11).
228. D'Onofrio, N., L. Servillo, and M.L. Balestrieri, *SIRT1 and SIRT6 Signaling Pathways in Cardiovascular Disease Protection.* Antioxid Redox Signal, 2018. **28**(8): p. 711-732.
229. Liszt, G., et al., *Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase.* J Biol Chem, 2005. **280**(22): p. 21313-20.
230. Haigis, M.C., et al., *SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells.* Cell, 2006. **126**(5): p. 941-54.
231. D'Onofrio, N., et al., *Sirtuins in vascular diseases: Emerging roles and therapeutic potential.* Biochim Biophys Acta, 2015. **1852**(7): p. 1311-22.
232. Borradaile, N.M. and J.G. Pickering, *Nicotinamide phosphoribosyltransferase imparts human endothelial cells with extended replicative lifespan and enhanced angiogenic capacity in a high glucose environment.* Aging Cell, 2009. **8**(2): p. 100-12.
233. Bao, X., et al., *Identification of 'erasers' for lysine crotonylated histone marks using a chemical proteomics approach.* Elife, 2014. **3**.
234. Frye, R.A., *Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins.* Biochem Biophys Res Commun, 2000. **273**(2): p. 793-8.
235. Houtkooper, R.H., E. Pirinen, and J. Auwerx, *Sirtuins as regulators of metabolism and healthspan.* Nat Rev Mol Cell Biol, 2012. **13**(4): p. 225-238.
236. Santos, L., C. Escande, and A. Denicola, *Potential Modulation of Sirtuins by Oxidative Stress.* Oxid Med Cell Longev, 2016. **2016**: p. 9831825.
237. Sack, M.N. and T. Finkel, *Mitochondrial metabolism, sirtuins, and aging.* Cold Spring Harb Perspect Biol, 2012. **4**(12).
238. Colak, Y., et al., *A potential treatment of non-alcoholic fatty liver disease with SIRT1 activators.* J Gastrointestin Liver Dis, 2014. **23**(3): p. 311-9.
239. Zhang, C.Y., et al., *Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes.* Cell, 2001. **105**(6): p. 745-55.
240. Bordone, L., et al., *Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells.* PLoS Biol, 2006. **4**(2): p. e31.
241. Moynihan, K.A., et al., *Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice.* Cell Metab, 2005. **2**(2): p. 105-17.
242. Imai, S. and L. Guarente, *Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases.* Trends Pharmacol Sci, 2010. **31**(5): p. 212-20.

243. Kitamura, Y.I., et al., *FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction.* Cell Metab, 2005. **2**(3): p. 153-63.
244. Hughes, K.J., et al., *FoxO1 and SIRT1 regulate beta-cell responses to nitric oxide.* J Biol Chem, 2011. **286**(10): p. 8338-8348.
245. Elchebly, M., et al., *Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene.* Science, 1999. **283**(5407): p. 1544-8.
246. Sun, C., et al., *SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B.* Cell Metab, 2007. **6**(4): p. 307-19.
247. Nogueiras, R., et al., *Sirtuin 1 and sirtuin 3: physiological modulators of metabolism.* Physiol Rev, 2012. **92**(3): p. 1479-514.
248. Kitada, M. and D. Koya, *SIRT1 in Type 2 Diabetes: Mechanisms and Therapeutic Potential.* Diabetes Metab J, 2013. **37**(5): p. 315-25.
249. Kitada, M., et al., *Sirtuins and Type 2 Diabetes: Role in Inflammation, Oxidative Stress, and Mitochondrial Function.* Front Endocrinol (Lausanne), 2019. **10**: p. 187.
250. Bagul, P.K. and S.K. Banerjee, *Insulin resistance, oxidative stress and cardiovascular complications: role of sirtuins.* Curr Pharm Des, 2013. **19**(32): p. 5663-77.
251. Waldman, M., et al., *Regulation of diabetic cardiomyopathy by caloric restriction is mediated by intracellular signaling pathways involving 'SIRT1 and PGC-1α'.* Cardiovasc Diabetol, 2018. **17**(1): p. 111.
252. Guo, R., et al., *SIRT1 suppresses cardiomyocyte apoptosis in diabetic cardiomyopathy: An insight into endoplasmic reticulum stress response mechanism.* Int J Cardiol, 2015. **191**: p. 36-45.
253. Erdal, N., et al., *Deterioration of bone quality by streptozotocin (STZ)-induced type 2 diabetes mellitus in rats.* Biol Trace Elem Res, 2011. **140**(3): p. 342-53.
254. Sadek, K.M., et al., *Spirulina platensis prevents hyperglycemia in rats by modulating gluconeogenesis and apoptosis via modification of oxidative stress and MAPK-pathways.* Biomed Pharmacother, 2017. **92**: p. 1085-1094.
255. Pallag, A., et al., *Comparative Study of Polyphenols, Flavonoids and Chlorophylls in Equisetum arvense L. Populations.* Revista de Chimie -Bucharest- Original Edition-, 2016. **67**: p. 530-533.
256. Commission, E.P., *European pharmacopoeia.* (No Title), 1969.
257. Lee, S., et al., *Blueberry Supplementation Influences the Gut Microbiota, Inflammation, and Insulin Resistance in High-Fat-Diet-Fed Rats.* J Nutr, 2018. **148**(2): p. 209-219.
258. Badale, A., et al., *Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats.* Farmacia, 2019. **67**(1): p. 92-98.
259. Pallag, A., et al., *Comparative Histo-Anatomical Researches on the Vegetative Organs and Assessment of Antioxidant Capacity of Two Species From Equisetum Genus.* Farmacia, 2016. **64**: p. 372-7.
260. Hegedűs, C., et al., *SIRT1 Activation by Equisetum Arvense L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats.* Molecules, 2020. **25**(11).
261. Avalos-Soriano, A., et al., *4-Hydroxyisoleucine from Fenugreek (Trigonella foenum-graecum): Effects on Insulin Resistance Associated with Obesity.* Molecules, 2016. **21**(11).
262. Narender, T., et al., *4-hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent.* Bioorg Med Chem Lett, 2006. **16**(2): p. 293-6.
263. Srichamroen, A., et al., *The Modifying Effects of Galactomannan from Canadian-Grown Fenugreek (Trigonella foenum-graecum L.) on the Glycemic and Lipidemic Status in Rats.* J Clin Biochem Nutr, 2008. **43**(3): p. 167-74.

264. Hamden, K., et al., *Inhibitory effect of fenugreek galactomannan on digestive enzymes related to diabetes, hyperlipidemia, and liver-kidney dysfunctions.* Biotechnology and Bioprocess Engineering, 2010. **15**(3): p. 407-413.
265. Della-Zuana, O., et al., *Acute and chronic administration of melanin-concentrating hormone enhances food intake and body weight in Wistar and Sprague-Dawley rats.* Int J Obes Relat Metab Disord, 2002. **26**(10): p. 1289-95.
266. Gomori, A., et al., *Chronic intracerebroventricular infusion of MCH causes obesity in mice. Melanin-concentrating hormone.* Am J Physiol Endocrinol Metab, 2003. **284**(3): p. E583-8.
267. Isaia, I., et al., *Comparative Analysis upon Action of Aqueous and Alcoholic Trigonella sp. Extracts on Fibroblasts Culture.* Revista de Chimie -Bucharest- Original Edition-, 2017. **68**: p. 562.
268. Lupascu, F., et al., *Biological evaluation of chitosan-antidiabetic drug formulations for the treatment of diabetes mellitus.* Farmacia, 2017. **65**: p. 508-514.
269. Marsh, D.J., et al., *Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism.* Proceedings of the National Academy of Sciences of the United States of America, 2002. **99**(5): p. 3240-3245.
270. Qu, D., et al., *A role for melanin-concentrating hormone in the central regulation of feeding behaviour.* Nature, 1996. **380**(6571): p. 243-7.
271. Siriwardhana, N., et al., *Modulation of adipose tissue inflammation by bioactive food compounds.* J Nutr Biochem, 2013. **24**(4): p. 613-23.
272. Arshadi, S., et al., *Evaluation of Trigonella foenum-graecum extract in combination with swimming exercise compared to glibenclamide consumption on type 2 Diabetic rodents.* Food & Nutrition Research, 2015. **59**.
273. Kiss, R., et al., *Insulin-Sensitizer Effects of Fenugreek Seeds in Parallel with Changes in Plasma MCH Levels in Healthy Volunteers.* Int J Mol Sci, 2018. **19**(3).
274. Kannappan, S. and C.V. Anuradha, *Insulin sensitizing actions of fenugreek seed polyphenols, quercetin & metformin in a rat model.* Indian J Med Res, 2009. **129**(4): p. 401-8.
275. Blaslov, K., et al., *Treatment approach to type 2 diabetes: Past, present and future.* World J Diabetes, 2018. **9**(12): p. 209-219.
276. Thrasher, J., *Pharmacologic Management of Type 2 Diabetes Mellitus: Available Therapies.* Am J Med, 2017. **130**(6s): p. S4-s17.
277. Maneze, D., et al., *"Some say no, some say yes": Receiving inconsistent or insufficient information from healthcare professionals and consequences for diabetes self-management: A qualitative study in patients with Type 2 Diabetes.* Diabetes Res Clin Pract, 2019. **156**: p. 107830.
278. Muhammad, S.A., et al., *Cellular Signaling Pathways in Insulin Resistance-Systems Biology Analyses of Microarray Dataset Reveals New Drug Target Gene Signatures of Type 2 Diabetes Mellitus.* Front Physiol, 2017. **8**: p. 13.
279. Ma, X., et al., *The Pathogenesis of Diabetes Mellitus by Oxidative Stress and Inflammation: Its Inhibition by Berberine.* Front Pharmacol, 2018. **9**: p. 782.
280. Wang, Y., et al., *The Role of HMGB1 in the Pathogenesis of Type 2 Diabetes.* J Diabetes Res, 2016. **2016**: p. 2543268.
281. Szabó, K., et al., *Fenugreek (Trigonella Foenum-Graecum) Seed Flour and Diosgenin Preserve Endothelium-Dependent Arterial Relaxation in a Rat Model of Early-Stage Metabolic Syndrome.* Int J Mol Sci, 2018. **19**(3).
282. Deyno, S., et al., *Efficacy and safety of cinnamon in type 2 diabetes mellitus and pre-diabetes patients: A meta-analysis and meta-regression.* Diabetes Res Clin Pract, 2019. **156**: p. 107815.

283. Bulboacă, A.E., et al., *Liposomal Curcumin is Better than Curcumin to Alleviate Complications in Experimental Diabetic Mellitus.* Molecules, 2019. **24**(5).
284. Bulboacă, A.E., et al., *Comparative Effect Of Curcumin Versus Liposomal Curcumin On Systemic Pro-Inflammatory Cytokines Profile, MCP-1 And RANTES In Experimental Diabetes Mellitus.* Int J Nanomedicine, 2019. **14**: p. 8961-8972.
285. Cao, Y., et al., *SIRT1 and insulin resistance.* J Diabetes Complications, 2016. **30**(1): p. 178-83.
286. Wang, R.H., et al., *Hepatic Sirt1 deficiency in mice impairs mTorc2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance.* J Clin Invest, 2011. **121**(11): p. 4477-90.
287. Milne, J.C., et al., *Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes.* Nature, 2007. **450**(7170): p. 712-6.
288. Luu, L., et al., *The loss of Sirt1 in mouse pancreatic beta cells impairs insulin secretion by disrupting glucose sensing.* Diabetologia, 2013. **56**(9): p. 2010-20.
289. Prud'homme, G.J., et al., *GABA protects pancreatic beta cells against apoptosis by increasing SIRT1 expression and activity.* Biochem Biophys Res Commun, 2014. **452**(3): p. 649-54.
290. Sulaiman, M., et al., *Resveratrol, an activator of SIRT1, upregulates sarcoplasmic calcium ATPase and improves cardiac function in diabetic cardiomyopathy.* Am J Physiol Heart Circ Physiol, 2010. **298**(3): p. H833-43.
291. Bugger, H. and E.D. Abel, *Molecular mechanisms of diabetic cardiomyopathy.* Diabetologia, 2014. **57**(4): p. 660-71.
292. Fishman, S.L., et al., *The role of advanced glycation end-products in the development of coronary artery disease in patients with and without diabetes mellitus: a review.* Mol Med, 2018. **24**(1): p. 59.
293. Kertész, A., et al., *Adverse impact of diet-induced hypercholesterolemia on cardiovascular tissue homeostasis in a rabbit model: time-dependent changes in cardiac parameters.* Int J Mol Sci, 2013. **14**(9): p. 19086-108.
294. Duan, J., et al., *Chikusetsu saponin IVa confers cardioprotection via SIRT1/ERK1/2 and Homer1a pathway.* Sci Rep, 2015. **5**: p. 18123.
295. Vahtola, E., et al., *Forkhead class O transcription factor 3a activation and Sirtuin1 overexpression in the hypertrophied myocardium of the diabetic Goto-Kakizaki rat.* J Hypertens, 2008. **26**(2): p. 334-44.
296. Yuan, Q., et al., *Advanced glycation end-products impair Na^+/K^+-ATPase activity in diabetic cardiomyopathy: role of the adenosine monophosphate-activated protein kinase/sirtuin 1 pathway.* Clin Exp Pharmacol Physiol, 2014. **41**(2): p. 127-33.
297. Thirunavukkarasu, M., et al., *Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase.* Free Radic Biol Med, 2007. **43**(5): p. 720-9.
298. Penumathsa, S.V., et al., *Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat.* J Mol Cell Cardiol, 2007. **42**(3): p. 508-16.
299. Juhasz, B., et al., *Cardioprotective effects of sour cherry seed extract (SCSE) on the hypercholesterolemic rabbit heart.* Curr Pharm Des, 2013. **19**(39): p. 6896-905.
300. Juhasz, B., et al., *Bromelain induces cardioprotection against ischemia-reperfusion injury through Akt/FOXO pathway in rat myocardium.* Am J Physiol Heart Circ Physiol, 2008. **294**(3): p. H1365-70.

9 Key words

9.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats

fenugreek, diosgenin, obesity, insulin resistance

9.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats

SIRT1; diabetes mellitus; insulin resistance; diabetic cardiomyopathy; *Equisetum arvense* L.; streptozotocin

10 List of publications

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/95/2024.PL
Subject: PhD Publication List

Candidate: Andrea Badale
Doctoral School: Kálmán Laki Doctoral School
MTMT ID: 10094334

List of publications related to the dissertation

1. Hegedűs, C., Muresan, M., **Badale, A.**, Bombicz, M., Varga, B., Szilágyi, A. T., Sinka, D. Z., Bácskay, I., Popoviciu, M., Magyar, I., Szarvas, M. M., Szőllősi, E., Németh, J., Szilvássy, Z., Pallag, A., Kiss, R.: SIRT1 Activation by Equisetum Arvense L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats.
   Molecules. 25 (11), 2541-2561, 2020.
   DOI: http://dx.doi.org/10.3390/molecules25112541
   IF: 4.411
2. **Badale, A.**, Pallag, A., Bombicz, M., Hegedűs, C., Kovács, D. K., Gulyás, H., Zdrîncă, M., Magyar, I., Marc, F., Németh, S., Kiss, R.: Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats.
   Farmacia. 67 (1), 92-98, 2019.
   DOI: http://dx.doi.org/10.31925/farmacia.2019.1.12
   IF: 1.607

DEBRECENI EGYETEM Egyetemi és Nemzeti Könyvtár

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

3. Kiss, R., Pesti-Asbóth, G., Szarvas, M. M., Stündl, L., Cziáky, Z., Hegedűs, C., Kovács, D. K., **Badale, A.**, Máthé, E., Szilvássy, Z., Gálné Remenyik, J.: Diosgenin and Its Fenugreek Based Biological Matrix Affect Insulin Resistance and Anabolic Hormones in a Rat Based Insulin Resistance Model.
Biomed Res. Int. 2019, 1-13, 2019.
DOI: http://dx.doi.org/10.1155/2019/7213913
IF: 2.276

Total IF of journals (all publications): 8,294
Total IF of journals (publications related to the dissertation): 6,018

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

14 March, 2024

DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

11 Acknowledgement

Words cannot express my gratitude to Supervisor, Assistant Professor Rita Kiss, MD, PhD, of the University of Debrecen, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, for knowledge, patience, guidance and moral support throughout my research journey.

I extend my sincere gratitude to the esteemed Rector, Professor Dr. Zoltán Szilvássy, for graciously permitting me to pursue my research endeavours under the auspices of the institution he leads.

I express my sincere appreciation to the Chairman of the defence committee, for graciously accepting this role, and to the esteemed members of the evaluation committee for generously sharing their knowledge and expertise, dedicating their time to assess my thesis.

I am equally grateful to Professor Annamaria Pallag of the University of Oradea, Faculty of Medicine and Pharmacy, Department of Pharmacy, for her invaluable advice, suggestions, and for providing botanical specimens crucial to my studies. I extend my thanks to the research assistants, laboratory aides, and co-authors whose contributions impacted and inspired my work.

Undoubtedly, my deepest gratitude is reserved for my family, particularly my parents, spouse, and children, whose unwavering belief in me has been a constant source of encouragement and motivation throughout this endeavour.

Furthermore, I wish to acknowledge the indispensable financial support received from the Higher Education Institutional Excellence Programme (NKFIH 1150-6/2019) of the Ministry of Innovation and Technology in Hungary, as part of the Therapeutic Purpose Development thematic program at the University of Debrecen, and the Thematic Excellence Programme of the Ministry for Innovation and Technology in Hungary (ED_18-1-2019-0028), within the framework of the University of Debrecen's thematic program. I am also grateful for the financial assistance provided by the Hungarian-European Research Infrastructure Network (EFOP-3.6.2-16-2017-00009) and the European Union and the State of Hungary under grant number GINOP-2.3.2-15-2016-00043, which have been instrumental in supporting my research endeavours.