SHORT THESIS 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

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Table of contents

1 Introduction 1

2 The involvement of select traditional herbal extracts such as fenugreek and horsetail in the prevention and treatment of obesity, T2DM and diabetic cardiomyopathy 3

2.1 Fenugreek (*Trigonella foenum-graecum* L.) 3

2.2 Horsetail (*Equisetum arvense* L.) 4

3 The role of SIRT1 in T2DM and DCM 6

4 Aims 8

5 Materials and Methods 9

5.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats 9

5.1.1 Ethics 9

5.1.2 Animals 9

5.1.3 Research Protocol 9

5.1.4 Induction of Obesity 9

5.1.5 Metabolic measurements 9

5.1.6 Determination of insulin sensitivity 10

5.1.7 Statistics 10

5.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats 11

5.2.1 Ethics 11

5.2.2 Animals 11

5.2.3 Research Protocol 11

5.2.4 Induction of Diabetes Mellitus 11

5.2.5 Microscopic examination of *Equisetum arvense* L. 12

5.2.6 Preparation of *Equisetum arvense* L. Extract 12

5.2.7 Determination of Phenolic Compounds from *Equisetum arvense* L using UPLC-DAD 12

5.2.8 Oral Glucose Tolerance Test (OGTT) 12

5.2.9 Insulin Tolerance Test (ITT) 12

5.2.10 Samples 12

5.2.11 Plasma insulin concentration 13

5.2.12 Western Blot 13

5.2.13 Statistics 13
6 Results 14
6.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats 14
6.1.1 The effects of diosgenin and fenugreek seed treatment on body weight 14
6.1.2 Effect of chronic diosgenin and fenugreek seed treatment on the weight of abdominal white adipose tissue 14
6.1.3 Effect of chronic diosgenin and fenugreek seed treatment on daily food intake 14
6.1.4 Effect of chronic diosgenin and fenugreek seed treatment on daily water intake 14
6.1.5 Effect of chronic diosgenin and fenugreek seed treatment on daily energy intake 14
6.1.6 Effect of chronic diosgenin and fenugreek seed treatment on insulin sensitivity 14
6.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats 15
6.2.1 Microscopic Examination of *Equisetum arvense* L. 15
6.2.2 Ultra-High Performance Liquid Chromatography (UHPLC) Analysis of *Equisetum arvense* L. Extract 15
6.2.3 Effect of *Equisetum arvense* L. on Body Weight 15
6.2.4 Effect of *Equisetum arvense* L. on Blood Glucose 15
6.2.5 Effect of *Equisetum arvense* L. on Glucose Tolerance 16
6.2.6 Effect of *Equisetum arvense* L. on Insulin Tolerance 16
6.2.7 Effect of *Equisetum arvense* L. on Fasting Plasma Insulin 16
6.2.8 Effect of *Equisetum arvense* L. on Adiposity 16
6.2.9 Effect of *Equisetum arvense* L. on Heart Weight Index 16
6.2.10 Effect of *Equisetum arvense* L. on SIRT1 Levels 16
7 Discussion 17
7.1 Fenugreek seed and its active agent diosgenin treatment effects on different metabolic parameters in rats 17
7.2 SIRT1 Activation by *Equisetum arvense* L. (Horsetail) Modulates Insulin Sensitivity in Streptozotocin Induced Diabetic Rats 19
8 Summary 22
9 Acknowledgement 23

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. 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. 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.

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

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

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. 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 phytochemical.

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.

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.

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. 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. Due to its anti-inflammatory properties, diosgenin has exhibited protective effects on the kidneys of diabetic rats, mitigating renal complications associated with T2DM.

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. 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.

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.

2.2 Horsetail (Equisetum arvense L.*)*

Equisetum arvense L, commonly known as field horsetail, is a perennial fern belonging to the Equisetaceae family.

Field horsetail thrives spontaneously in light sandy soils and is distributed across various regions including Europe, Africa, South America, Southern Asia, Turkey, and Iran.

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.

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. 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.

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. 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.

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. 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. 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.

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.

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. 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.

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. 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.

3 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.

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.

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.

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. 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, 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. In SIRT1-KO mice, the quantity of UCP2 protein was increased, while serum insulin levels were low. Conversely, UCP2-KO mice showed increased insulin secretion and ATP production. 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.

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. 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. 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.

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.

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.

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. Recent investigations further 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.

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.

Calorie restriction (CR) has emerged as a promising strategy for delaying the onset of various age-related diseases, including T2DM and DCM. SIRT1, known to be upregulated by calorie restriction (CR), is closely associated with the anti-aging effects observed during CR. 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.

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.

4 Aims

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.

5 Materials and Methods

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

5.1.1 *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.

5.1.2 *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.

5.1.3 *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.

5.1.4 *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, 1 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. 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.

5.1.5 *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.

5.1.6 *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.

5.1.7 *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.

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

5.2.1 *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.

5.2.2 *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).

5.2.3 *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

5.2.4 *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. 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.

5.2.5 *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. 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.

5.2.6 *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.

5.2.7 *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.

5.2.8 *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. 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.

5.2.9 *Insulin Tolerance Test (ITT)*

In week 5, an insulin tolerance test (ITT) was conducted according to a method optimized and validated within our institute. 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.

5.2.10 *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.

5.2.11 *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%.

5.2.12 *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[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).

5.2.13 *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.

6 Results

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

6.1.1 *The effects of diosgenin and fenugreek seed treatment on body weight*

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.

6.1.2 *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. 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. Conversely, low dosages of diosgenin and TFG seeds were insufficient to counteract the effects of HFD.

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

It was observed that all HFD groups consumed significantly less food compared to the control group.

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

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.

6.1.5 *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.

6.1.6 *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 group. 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.

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

6.2.1 *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. 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).

6.2.2 *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. 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.

6.2.3 *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. 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.

6.2.4 *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. 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.

6.2.5 *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, indicating reduced glucose tolerance. However, a significant improvement was observed only in the 100HT group compared to both the DC and *Equisetum arvense* L. treated animals.

6.2.6 *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, indicating decreased glucose tolerance. However, a notable improvement was observed only in the 100HT group.

6.2.7 *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.

6.2.8 *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. 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.

6.2.9 *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. A statistically significant increase in heart weight index was observed in the DC and 200HT groups.

6.2.10 *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. 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.

7 Discussion

7.1 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. 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.

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. 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.

7.2 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. 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.

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. 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. 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, whereas SIRT1 activation can mitigate hepatic IR in the context of obesity. Within adipocytes, SIRT1 promotes GLUT4 translocation and subsequent glucose uptake. Additionally, SIRT1 is expressed in pancreatic ß-cells, where it augments glucose-stimulated insulin secretion. Studies indicate that selective upregulation of SIRT1 in ß-cells enhances glucose metabolism in mice, whereas its selective deletion leads to glucose intolerance. Furthermore, data suggests that SIRT1 confers protection against ß-cell apoptosis.

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. 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. 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. 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. 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, and deacetylate p53, thereby reducing apoptosis. 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. 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.

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. Elucidating the specific targets and mechanisms underlying this effect will necessitate further experimentation.

8 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) 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.

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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 *