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Effects of Hesperidin on Histopathological and Epigenetic Changes in Streptozotocin-Induced Type-2 Diabetic Rats

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Abstract

Chemicals have been shown to induce epigenetic changes that alter glucose metabolism genes, potentially leading to insulin resistance and increasing the risk of metabolic disorders like type 2 diabetes. This study was aimed to assess histopathological and epigenetic changes in insulin signalling molecules in STZ-induced type-2 diabetic rats and the possible therapeutic role of hesperidin. Hesperidin (100mg/kg b.wt) was administered to STZ-induced rats and assessed for its protective role and epigenetic mechanisms in the gastrocnemius muscle. Diabetic rats exhibited significant increase ($p<0.05$) in renal function markers such as urea (60, 140, 80, 70, and 79 mg/dL) and creatinine (0.9, 2, 1.2, 1.1, and 1.0 mg/dL), oxidative stress markers, while antioxidant enzymes such as superoxide dismutase (0.9, 0.5,0.8, 0.87 and U/mg protein) and catalase (1, 0.4,0.86, 0.92 and 1.13 U/mg protein) were markedly lower ($p<0.05$). Histopathological analysis revealed a decrease and disruption in muscle fibres. The mRNA expression of insulin signalling molecules PI3K (1, 0.6, 0.8, 1.1, and 1 fold) and Munc18 (1, 0.6, 0.8, 1, and 0.9) was significantly ($p<0.01$) reduced in diabetic groups. Epigenetic studies showed CpG island methylation in the promoter regions of GLUT4, Akt, and IR genes in diabetic rats. However, hesperidin treatment restored the detrimental changes caused by diabetogenic agent, streptozotocin. The present study concludes that hesperidin plays a central role in regulating epigenetic mechanisms of insulin signalling molecules and GLUT4 translocation in skeletal muscle and thereby protects the muscle cells.

Keywords: *DNA Methylation, Diabetes, Epigenetics, Hesperidin, Health and Well-Being, Insulin Signalling, Novel Methods.*

Introduction

The pervasive sickness in the current era, diabetes is a leading cause of cardiovascular disease (CVD), a major global health concern. Oxidative stress in skeletal muscles is caused by obesity and hyperglycemia. It has had a major detrimental impact on human life and health economics, spreading to pandemic proportions around the globe. As a result, the

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development of novel, cutting-edge drugs that lessen these harmful events ought to be given top priority [1]. According to statistics from around the world, approximately 74% of people are unable to purchase allopathic medicine's products. As a result, they must rely on traditional remedies, which are mostly made from plants. Hence, we study the common bioflavonoid Hesperidin as the drug to identify its effect on Diabetics. The objective of this experimental investigation is to identify and evaluate the therapeutic properties of hesperidin, a bioflavonoid derived from orange peel (a citrus fruit), on the molecules that regulate insulin signal in the gastrocnemius (skeletal) muscle hyperglycemia-instigated type-2 diabetes (T2DM) rats [2]. Because it targets apoptotic pathways and induces apoptosis, β-sitosterol has a great therapeutic potential as an anticancer treatment against oral cancer KB cells [3]. Hesperidin is a bioflavonoid (flavanone glycoside) with hypolipidemic and hypoglycemic hypoglycemic properties that are abundant in oranges and lemons. Hesperidin, one of several biophenols, is important as a naturally occurring medicinal substance [4,5]. The majority of citrus plants contain hesperidin, a powerful vitamin that animals consume through diet. This study aims to determine and assess the potential therapeutic effects of hesperidin, a bioflavonoid derived from orange peel, on the molecules that control insulin signal in the skeletal muscle of obese rats with hyperglycemia-induced type- 2 diabetes (T2DM) [6]. Oranges and lemons are rich in hesperidin, a bioflavonoid (flavanone glycoside) having hypolipidemic and hypoglycemic qualities. Among the several biophenols, hesperidin is significant as a naturally occurring medication [6]. Higher caries severity, higher DMFT scores, and a plaque ecology that favors Streptococcus mutans are all associated with the presence of H. pylori in severe carious lesions [7]. Hesperidin, a potent vitamin that animals get from eating, is found in most citrus trees. It is appropriately characterized by the European Food Safety Authority (EFSA) to take hesperidin along with diosmin, troxerutin, and other drugs. The intended outcome is the maintenance of normal venous-capillary permeability, which has advantageous physiological implications [8].

In this study, we examine and investigate some of the therapeutic effects of hesperidin against type-2 diabetes using diabetic insulin resistance [9,10]. In different stages of OSCC, NGS analysis identifies a variety of genetic alterations that help with minimal intervention techniques and individualized treatment plans [11]. The majority (80%) of the circulating glucose is absorbed by skeletal muscle following a postprandial meal before being taken up by glucose transporters into the cell. The study of epigenetics focuses on how environmental factors and behaviour can alter gene expression [12**,** 13]. While epigenetic modifications can alter how your body interprets a DNA sequence, they are reversible and do not alter your DNA sequence like genetic modifications do [14,15]. Through its inhibition of HSC-3 oral squamous carcinoma cells' proliferation, migration, invasion, and aerobic glycolysis, calotropin has anti-cancer effects [16]. Metabolic illnesses like diabetes and obesity are primarily caused by genetic alterations, but they can also be exacerbated by epigenetic changes brought on by a sedentary lifestyle and ecological stressors in response to imbalances in energy expenditure and consumption. Insulin resistance can result from increased synthesis of non-coding RNAs, histone modifications, and DNA methylation that reduces the transcriptional function of key beta-cell genes. In addition to inflammation linked to obesity, elevated Reactive Oxygen Species (ROS), and damage to DNA in many body regions, epigenetics plays a role in the expression of the underlying gene networks that cause insulin resistance and insufficiency.

Loss of body weight persisted even after serum lipid levels decreased in STZ administered diabetic mice & the modulatory action of biotransformation enzymes was the main cause. Trained immunity is based on epigenetic regulation of histone changes that cause chromatin to open permanently. A possible marker for early detection and prognosis, elevated salivary MMP-9 levels are correlated with the severity of OSCC and malignant transformation [17].

Materials and Methods

Chemicals and Reagents

Hesperidin and streptozotocin were purchased from Sigma-Aldrich (St. Louse, MO, USA). Primers specific to genes such as GLUT 4, IR, IRS-1, Akt, and β-actin were obtained from Eurofins Genomics India Pvt Ltd. in Bangalore, India. The remaining chemicals and reagents needed for the investigation were obtained from the following sources: Sisco Research Laboratories, India; MP Biomedicals, LLC; 9 Goddard, Irvine, CA-92618, USA; New England BioLabs (NEB), USA; and Promega (USA).

Animals

We acquired adult male Wistar albino rats, weighing between 150 and 180 days, from MASS Biotech in Chennai, India. The rats were housed at the Central Animal House, Saveetha Dental College and Hospital in Chennai, Tamil Nadu, under standard environmental conditions that included a constant 12-hour light/dark cycle, standard temperature and specific humidity (21 ± 2 ◦C), regular pellet meal, and unlimited water, according to the Institutional Animal Ethical Committee. The current work was approved by adhering to the current guidelines (IAEC No.: BRULAC/SDCH/SIMATS/IAEC/04-2022/101, dated 2 April 2022).

Experimental Design

Group 1: Comprised of control rats in good health; Group 2: Diabetic rats induced by streptozotocin; Group 3: Diabetic rats treated with hesperidin at a dose of 100 mg/kg bwt for 30 days; Group 4: Diabetic rats treated with metformin at a dose of 50 mg/kg bwt for 30 days; Group 5: Diabetic rats treated with hesperidin. Hesperidin was dissolved in 1mL of 0.9% saline and then administered orally via an intragastric tube at different doses for 30 days. One milliliter of distilled water was used to dissolve the standard medicine, metformin [22]. Sodium thiopentone (40 mg/kg body weight) was used to sedate the animals on the final day of the experiment. Blood was extracted using heart puncture, and the sera were separated and kept at -80 ◦C. immediately after being dissected, the gastrocnemius muscle was measured for the following parameters.

Histopathological Study

The gastrocnemius muscle wad stained with hematoxylin and eosin dye after being histopathologically inspected and stored in paraffin using formalin (10% neutral buffered) [23]. The x200 magnification semi-thin sections produced by the LKB ultra-microtome were identified with a “Olympus” light microscope fitted with a “Nikon” digi-cam.

Methylation Specific PCR

The first CpG islands were discovered around the Glut4 promoter using the Methprimer software, which ensured a minimum 50% GC content and a subtracted CpG to anticipated CpG ratio >0.6. Methyl Primer Express software was utilized to generate methylation-specific primers, and Table 4 gives details about the primer sequences. After that, genomic DNA was extracted from the gastrocnemius muscles and measured in order to evaluate its quality. EZ DNA Methylation Kit was used to bisulfite modify the isolated DNA. The model utilized

for MSP was the naked DNA that had undergone bisulfite modification [24].

A 0.2 mM deoxynucleotide triphosphate (dNTP), 3 mM magnesium chloride ($MgCl_2$), 0.2 mM methylation-specific and unmethylation-specific primers, 1 U HotStarTaq DNA Polymerase, and 2 µl bisulfite-administered DNA were combined to create the PCR mix. Molecular biology grade water was used to adjust the overall capacity to 20 µl. Both positive and negative controls were present. Water was used as the +ve control since it contained no DNA template, and CpG methyltransferase from New England Biolabs was used to methylate hypermethylated rat DNA in vitro. List of primers used in this study is given in table1.

Table1. List of Gene Specific Primers Used in this Study

Name of the gene	**Primer sequence**	**Product (bp)**	**Annealing temperature (°C)**
Glut 4 (Methylated)	Sense primer: 5′-GAT GGG TCG TAG ATT GTG TAT CG-3′ Anti-sense primer: 5′-ACC TTA AAA AAT CCG CGA CTC GC-3′	121	59
Glut 4 (Unmethylated)	Sense primer: 5′-GGG ATG GGT TGT AGA TTG TGT ATT-3′ Anti-sense primer: 5′-AAC CTT AAA AAA TCC ACA AC-3′	119	58
Insulin receptor (IR) (Methylated)	Sense primer: 5′- TTG TTT TAG ATT TTA TAT AAC GCG A-3′ Anti-sense primer: 5′- TAA AAA AAA CAC ACA AAC CTC GAC-3′	107	58
Insulin receptor (IR) (Unmethylated)	Sense primer: 5′- TTG TTT TAG ATT TTA TAT AAT GTG A-3′ Anti-sense primer: 5′- TAA AAA AAA CAC ACA AAC CTC AAC-3′	109	59
Akt (Methylated)	Sense primer: 5′- TTA AGG ACG GTG TTA TTA TGA AGA C-3′ Anti-sense primer: 5′-TCT CCT CCA TTA AAA TAA ACT CGA A-3′	112	57
Akt (Unmethylated)	Sense primer: 5′-AAG GAT GGT GTT ATT ATG AAG ATG T-3′ Anti-sense primer: 5′-TCT CCT CCA TTA AAA TAA ACT CAA A-3′	118	59

Statistical Analysis

Using computer-based software (SPSS 7.5 using Windows student version), the data were statistically analyzed using one-way analysis of variance and Duncan's multiple comparison tests to determine the significance of individual changes between the control and treatment groups. A significance threshold of $p<0.05$ was used to the analysis.

Results

Effect of Hesperidin on kidney Function Markers in STZ-Induced Type-2 Diabetic Rats

Figure 1A and 1B show the effect of hesperidin on urea and creatinine respectively. While comparing between the control and treated group, we could notice that both Urea and Creatinine levels show significant increase in Diabetic group than the diabetes with Hesperidin. Treated and Diabetes with Metformin treated and control groups.

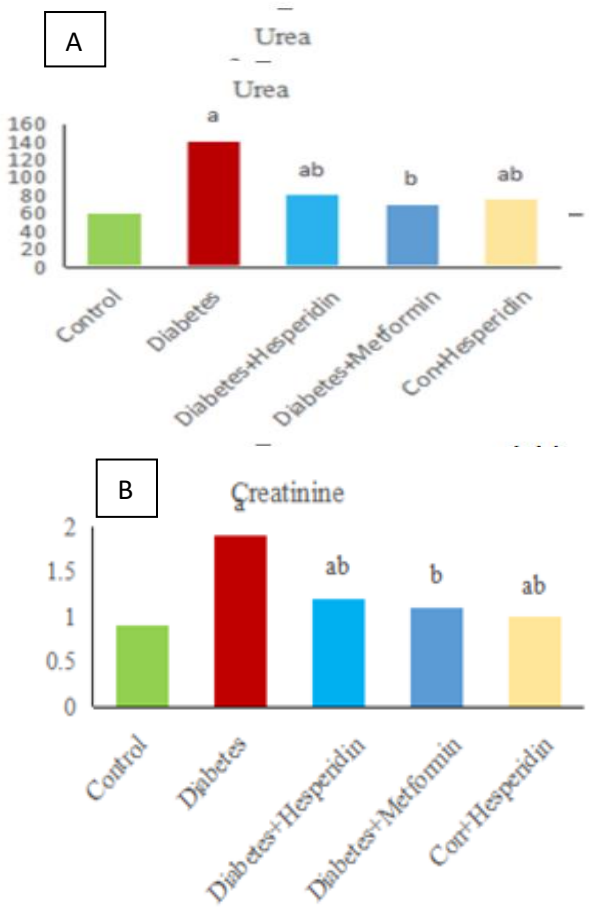


Figure 1. Hesperidin on Kidney Function Markers

Effect of Hesperidin on Oxidative Stress Markers H_2O_2 and LPO in the Gastrocnemius Muscle of STZ-Induced Type-2 Diabetic Rats

Figure 2A and 2B **s**how the effect of Hesperidin on H_2O_2 and LPO respectively. While comparing between the control and treated group, we could notice that both H_2O_2 and LPO levels show significant increase in Diabetic group than the diabetes with Hesperidin. Treated and Diabetes with Metformin treated and control groups.

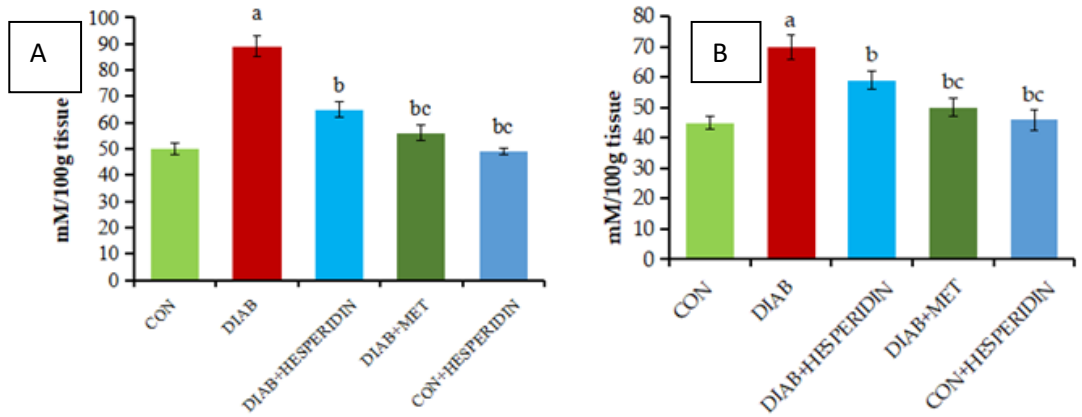


Figure 2. Hesperidin on H_2O_2 and LPO Activity

Effect of Hesperidin on Antioxidant Enzymes (SOD and CAT) in the Gastrocnemius Muscle of STZ-Induced Type-2 Diabetic Rats

Figures 3A and 3B show the effect of Hesperidin on antioxidant enzymes (SOD and CAT) respectively. While comparing the control and treated group, we could notice that both SOD and CAT levels show a significant increase in the Diabetic group than diabetes with Hesperidin Treated and Diabetes with Metformin treated and control groups.

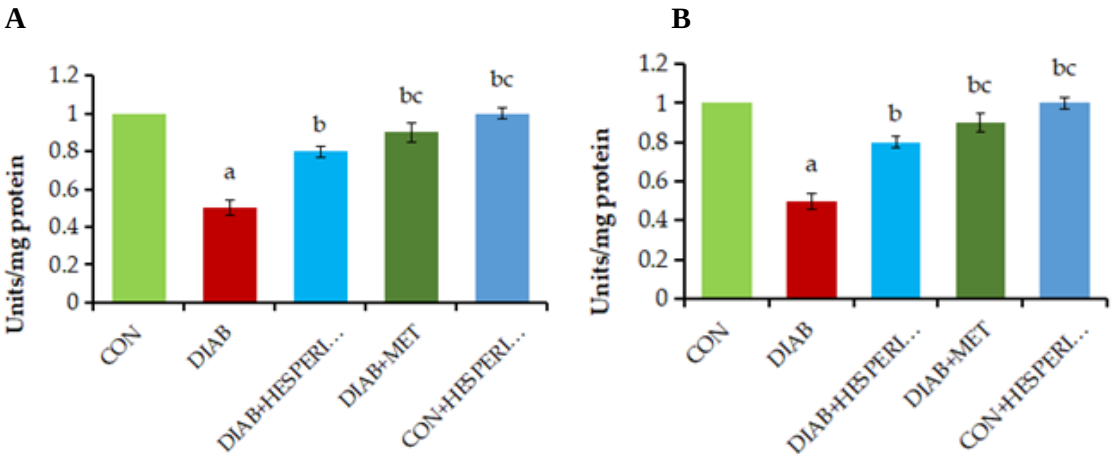


Figure 3. Hesperidin on SOD and CAT Activity.

Effect of Hesperidin on PI3K mRNA Expression in the Gastrocnemius Muscle of STZ-Induced type-2 Diabetic Rats

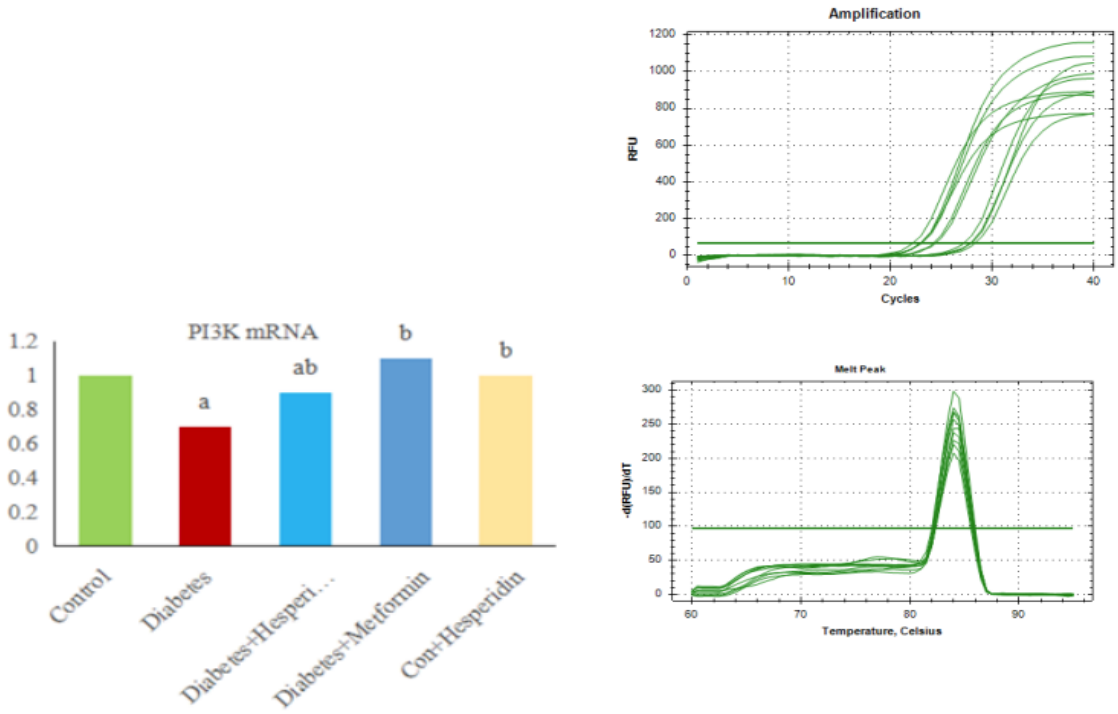


Figure 4. Hesperidin on PI3K .mRNA Expression in Skeletal Muscle

In figure 4, the PI3K mRNA expression is studied in the skeletal muscle and the Amplification chart shows increased value with 30 to 40 cycles. The melting peak shows at 85 degree Celsius and the untreated group shows lower value of diabetes.

Effect of Hesperidin on Munc 18 mRNA Expression in the Gastrocnemius Muscle of STZ-Induced Type-2 Diabetic Rats

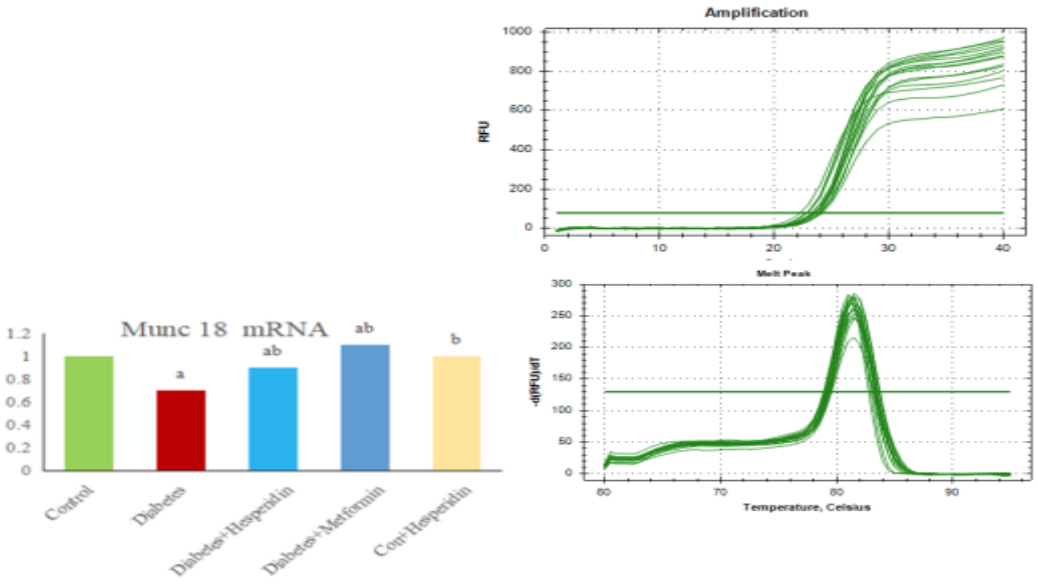


Figure 5. The Untreated Groups show a Lower Value of Diabetes than the Treated and Control for Munc18 mRNA Expression in Skeletal Muscle

In Figure 5, the Munc 18 mRNA expression in skeletal muscle is studied and the Amplification chart shows an increased value with 30 to 40 cycles and a melting peak at 80 degrees Celsius and the untreated groups show lower value of diabetes [22].

Effect of Hesperidin on Histopathological Changes in the Gastrocnemius Muscle of STZ-Induced Type-2 Diabetic Rats.

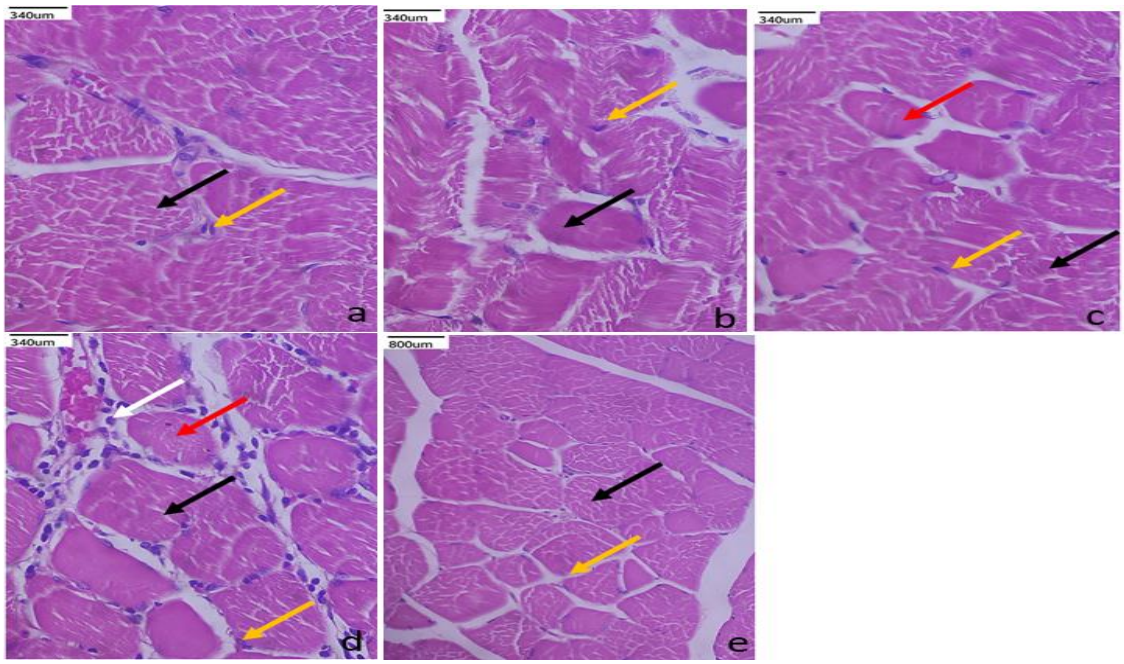


Figure 6a-e. Histopathological Study of Skeletal Muscle

The normal appearing skeletal muscle with black myofibrils and myonuclie is shown as yellow arrow in figure 6a and b. Figure 6b shows the normal appearing skeletal muscle with focal atrophy (red arrow) noted after the induction of STZ. In part d of figure 6, along with normal appearing skeletal muscle (black- myofibrils) and myonuclei (yellow) mild atrophy is noted (red arrow) and chronic inflammation in the intermuscular connective tissue (white). After the treatment of Hesperidin, normal-appearing skeletal muscle (black- myofibrils) and myonuclei (yellow) is observed.

Epigenetic Study

Using methyl-specific PCR, DNA methylation patterns were investigated, with an emphasis on CpG islands. Gene promoters are often the locations of CpG islands, regions of the genome where CpG dinucleotides accumulated and employ epigenetic regulation to modify gene expression. Methyl-specific PCR is a technique used by researchers to evaluate the methylation state of CpG islands by amplifying specific DNA sequences that are methylated or unmethylated. This technique advanced our understanding of the role of epigenetic modifications and gene regulation by illuminating the connection between DNA methylation and gene activity through the analysis of CpG island methylation.

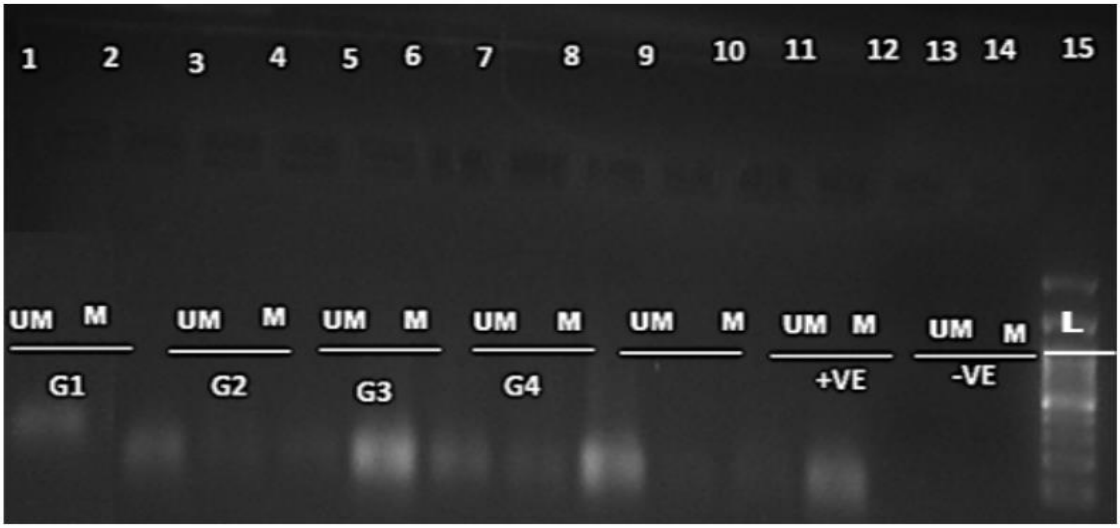
In this study, CpG island methylation was found in the control and STZ-treated diabetic rats' promoter regions of the GLUT4, Akt, and IR genes [23]. The results showed that the group was activated by STZ. Notably, hesperidin administration markedly reduced methylation levels in the CpG islands of the GLUT4, Akt, and IR genes relative to the diabetic control animals (as illustrated in the photos from fig. 7-8). These findings show that hesperidin can protect these gene promoters from the harmful effects of methylation.

IR

G1- Control
G2- Control+ Hesperidin
G3- STZ diabetes
G4-STZ+ Hesperidin
G5- STZ+ metformin

Figure 7. Identification of Methylated and Non-Methylated ir Gene**.**

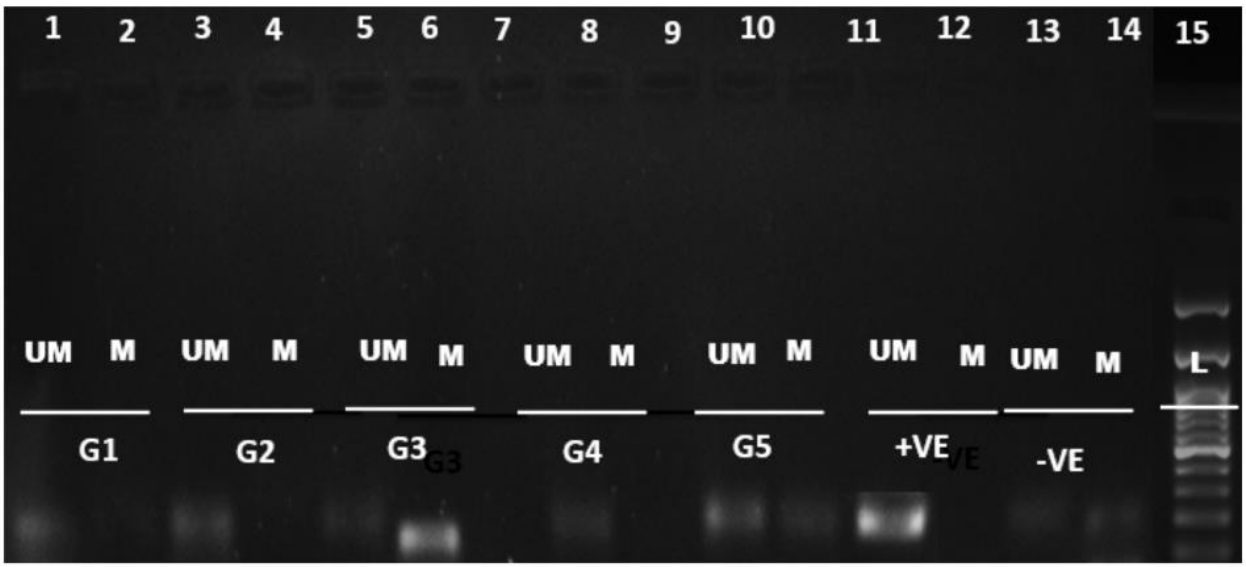
Akt



G1- Control
G2- Control+ Hesperidin
G3- STZ diabetes
G4-STZ+ Hesperidin
G5- STZ+ metformin

Figure 8. Identification of Methylated and Non-Methylated Akt Gene

GLUT4



G1- Control
G2- Control+ Hesperidin
G3- STZ diabetes
G4-STZ+ Hesperidin
G5- STZ+ metformin

Fig. 9. Identification of Methylated and Non-Methylated glut4 Gene

Discussion

Experimental rats induced with streptozotocin that induce hyperglycemia have been widely used as an effective vehicle to investigate the effects of various hypoglycemic medications [24]. When exposed to STZ, pancreatic beta cells are both adversely affected by O2-radicals and possibly by hydroxyl radicals [17]. The mechanism by which streptozotocin induces diabetes including selective destruction of pancreatic b-cells through DNA alkylation was explained [18]. For the early identification and management of OPMDs, miRNAs exhibit potential as biomarkers and therapeutic targets [25].

In diabetic rats' hearts and kidneys, hyperglycemia caused histone H3 to become deacetylated and dephosphorylated. Moreover, in hyperglycemic/hyperinsulinaemic circumstances, Fbn1 and Col3A1 mRNA expression rose in the kidneys and decreased in the heart. Chromatin immunoprecipitation also revealed an increase in the degree of histone H3 acetylation of the Fbn1 gene, but not the Col3A1 gene, which is comparable to mRNA expression. Anil et al indicated that the Fbn1 gene's change in expression is epigenetically regulated, but the Col3A1 gene's expression may be affected by other histone modifications or may not be affected by epigenetic regulation at all [26]. They also gave the first proof of the impact of hyperglycemia/hyperinsulinemia on histone H3 alterations, which may change the expression of genes involved in the extracellular matrix. The growing use of genome-wide DNA methylation profiles as a biomarker to identify individuals who may be at risk of diabetes or certain related problems [27]. Creating a prediction model that considers both DNA methylation alterations and genetic information could be a useful diagnostic strategy for all forms of diabetes and could result in further novel treatments. Leukoplakia, OSMF, and OSCC patients may be evaluated for the likelihood of malignant transformation using circulating exosomal miRNAs miRNA 21, miRNA 184, and miRNA 145 as possible biomarkers [28].

The prevalence of diabetes has increased and is on the rise in recent decades, and it will continue to Change in the levels and disturbances in the generation and uptake of glucose, insulin secretion, insulin action, and insulin resistance may lead to illness. It appears that hormonal interactions and various stressors may negatively affect this condition. One of the main causes of T2DM and the main issue related to obesity and a metabolic condition is peripheral insulin resistance. Insulin resistance can be caused by impaired insulin signalling, numerous post-receptor intracellular flaws, including impaired glucose transport, glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis, as well as decreased insulin-incited glucose uptake in skeletal muscle. Due to its numerous causes, diabetes is a serious medical condition.

In this study, we focused on evaluating the anti-diabetic role of Hesperidin in the streptozotocin-induced T2DM, and Hesperidin can enhance the insulin sensitivity in the skeletal muscles of diabetic rats. MiRNA which are non-coding RNAs epigenetically modify the phenotype and hence we conclude that it has epigenetic role as well. Eating a diet heavy in fat causes diabetes mellitus, which gradually damages the liver, skeletal muscle, and adipose tissue—organs involved in metabolism. Hyperglycemia is the outcome, with a drop in HDL and an increase in glucose, cholesterol, triglycerides, and LDH. Hyperinsulinemia and dysregulated insulin production are intimately linked to persistently high blood insulin levels in type 2 diabetes [29]. According to a correlation between salivary 1-25dihydroxycholecalciferol and IL-17A levels throughout orthodontic treatment stages, vitamin D administration may hasten

tooth movement with little harm to surrounding tissue [30].

Streptozotocin induction alters beta cell activity and leads to an aberrant structure of insulin that ultimately prevents it from binding to insulin receptors in target organ cells. This may open the door to lessening the insulin receptor's ability to bind to insulin receptor substrates, which in turn may lessen the activity of downstream signalling pathways like PI3K, Akt, and AS 160. Reduced GLUT4 transporter translocation ultimately results in decreased glucose absorption and decreased glucose metabolism [31]. To maintain normal blood glucose levels, insulin normally controls glycolysis and gluconeogenesis, which starts the uptake and oxidation of glucose in peripheral organs including muscle and adipose cells [32]. Under diabetes conditions, alterations in glucose oxidation in metabolic organs lead to frequent fluctuations in the activity of enzymes associated with these pathways, which in turn cause insulin resistance in these organs [33]. The two main enzymes involved in gluconeogenesis are fructose-1,6 bisphosphatase and glucose-6-phosphatase. Since insulin normally acts as a suppressor of gluconeogenic enzymes, the state of insulin shortage is the cause of the activation of these enzymes. Higher blood glucose levels result from increased gluconeogenic enzyme activity in type 2 diabetes (T2DM) since it creates hydrogen and mixes with NADP+ to form NADPH and enhance lipogenesis [34]. In our investigation, we observed that the gastrocnemius muscles of diabetic rats had considerably higher amounts of fructose-1,6 bisphosphonates and glucose-6-phosphatase. In this study, we found that giving hesperidin to experimental rats with high-fat diets and streptozotocin-induced type 2 diabetes increased their insulin sensitivity in the skeletal muscle. By increasing the activity of glucokinase, phosphorylating insulin receptor (IR) and phosphoinositide-dependent kinase 1 (PDK1), and lowering the activity of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver, hesperidin has been found to regulate glycolysis and gluconeogenesis. Hesperidin boosted glucose absorption in primary rat adipocytes in a cell-based experiment. By stimulating the IR/PDK1 pathway, it is noted that the powerful preventative impact of hesperidin against HFD-induced insulin resistance. Glycolysis generally affects insulin secretion as well as several cell metabolic activities. Key glycolysis enzymes HK and PK deficiencies can cause decreased glycolysis as well as decreased absorption and utilization of glucose for energy production, which can result in insulin resistance [35]. Due to defective insulin signalling, HK and PK were reduced in diabetic rats produced with streptozotocin and a high-fat diet in the current study. When compared to the metformin group, the treatment of Hesperidin increased the levels of these glycolytic enzymes in the skeletal muscle of diabetic rats. The phytocompound coumarin likewise exhibited a comparable characteristic, raising the amounts of glycolytic enzymes in diabetic rats relative to the control group [36]. Similarly, increased levels of glycolytic enzymes were demonstrated in the skeletal muscle of diabetics due to flavonoids [37].

Hesperidin therapy improved HG-induced insulin resistance by decreasing oxidative stress and mitochondrial dysfunction, in part by inhibiting the silencing of miR-149 mediated by DNMT1 [38]. Our study showed a significant increase in methylation levels in the STZ-induced diabetic group compared to the control group, indicating the impact of diabetes on the methylation of these specific gene promoters. This hypermethylation suggests a potential disruption in insulin signaling and impaired glucose metabolism associated with diabetes.

Insulin signaling, mostly through its effects on metabolic organs, is essential for regulating several physiologic processes, including

glucolipid homeostasis [39]. Insulin binding to the insulin receptor causes β-subunit tyrosine autophosphorylation, which phosphorylates additional substrates and initiates a signalling cascade to oxidize glucose for energy production. Insulin resistance may result from disruptions in various signalling pathways [40]. By altering glucose and lipid homeostasis, a high-fat diet interferes with insulin signalling, altering the usual functioning of insulin signalling molecules and leading to insulin resistance [41]. In particular, insulin signalling molecule change may be more pronounced in skeletal muscle—where 80% of glucose oxidation occurs—than in other organs including the liver and adipose tissue.

Insulin signalling pathways heavily rely on IRS-1, which is exclusive to peripheral tissues like skeletal muscle and adipose tissue. Diabetes results from inefficient insulin binding to the insulin receptor, which causes IRS-1 to be phosphorylated on the serine rather than the tyrosine kinase, which reduces IRS-1 activation and reduces the activity of downstream insulin signaling molecules like PI3 kinase, Akt, and AS160 [42]. The activity of Hesperidin on insulin signalling molecules, such as IRS-1, in the skeletal muscle of rats with high-fat diets and streptozotocin-induced rats shows that in comparison to the control group, the diabetic group had decreased levels of IRS-1 mRNA. Reduced activity of IRS-1 could result from changes in gluconeogenic and glycolytic processes.

Reduced binding of the insulin receptor to IRS-1 may result from modified gluconeogenic and glycolytic enzyme activity, which in turn may have led to diminished IRS-1 activity. As a result, there was less Akt activation. In diabetic skeletal muscle, study by Zang et al revealed that fucoxanthin treatment increased IRS-1 mRNA levels [43]. In T2DM skeletal muscle, Folium Mori led to increased levels of IRS-1 gene expression [44]. When compared to metformin, the Hesperidin therapy increased IRS-1 levels and demonstrated the mechanism behind its antidiabetic effects. We also observed the Effect of hesperidin on kidney function markers in STZ-induced Type-2 diabetic rats where the effect of Hesperidin on Urea and Creatinine were noted respectively. Both Urea and Creatinine levels show a significant increase in the Diabetic group than the diabetes with Hesperidin treated and Diabetes with Metformin treated and control groups [45].

We noted the Effect of hesperidin on oxidative stress markers H_2O_2 and LPO in the gastrocnemius muscle of STZ-induced Type-2 diabetic rats. By comparing the control and treated group, we noted that both H_2O_2 and LPO levels show a significant increase in the Diabetic group than the diabetes with Hesperidin treated and Diabetes with Metformin treated and control groups. We noted the Effect of hesperidin on antioxidant enzymes (SOD and CAT) in the gastrocnemius muscle of STZ-induced Type-2 diabetic rats. By doing a comparison between the control and treated group, we could notice that both SOD and CAT levels show a significant increase in the Diabetic group than diabetes with Hesperidin Treated and Diabetes with Metformin treated and control groups [46]. We also studied the Effect of hesperidin on Munc 18 mRNA expression in the gastrocnemius muscle of STZ-induced Type-2 diabetic rats. The Untreated groups show a lower value of Diabetes than the treated and control for Munc18 mRNA expression in skeletal muscle. Increased IRS-1 degradation was shown in the fatty tissue of a type -2 diabetic mouse model by Wang et al., who also proposed that impaired glucose uptake was caused by malfunctioning GLUT4 [47].

Using methyl-specific PCR, DNA methylation patterns were investigated, with an emphasis on CpG islands. Gene promoters are often the locations of CpG islands, regions of the genome where CpG dinucleotides

accumulate and employ epigenetic regulation to modify gene expression. Methyl-specific PCR is a technique used by researchers to analyse the methylation state of CpG islands by amplifying specific DNA sequences that are methylated or unmethylated. This technique advanced our understanding of the role of epigenetic modifications and gene regulation by illuminating the connection between DNA methylation and gene activity through the analysis of CpG island methylation.

In this investigation, the promoter regions of the GLUT4, Akt, and IR genes in diabetic rats treated with STZ and the control group showed CpG island methylation. The outcomes demonstrated that STZ stimulated the group. Compared to the diabetic control rats, the injection of hesperidin significantly lowered the methylation levels in the CpG islands of the GLUT4, Akt, and IR genes.

Conclusion

The current investigation shows that hesperidin regulates the expression of GLUT 4 translocation signalling molecules in gastrocnemius muscle and insulin signalling molecules (IR/IRS-1/Akt/GLUT4), hence playing a major role in glucose homeostasis. Hesperidin regulates STZ-mediated hypermethylation of insulin signalling molecules in the gastrocnemius muscle, according to epigenetic research. Hesperidin may therefore be used as a therapeutic natural medication to treat type 2 diabetes.

Conflict of Interest

The authors hereby declare that there is no conflict of interest.

Acknowledgement

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References

[1]. Herman, W. H., 2017, The Global Burden of Diabetes: An Overview. 10.1007/978-3-319-41559-8_1.

[2]. Pyrzynska, K., 2022, Hesperidin: A Review on Extraction Methods, Stability and Biological Activities. *Nutrients*, 14(12), 2387. doi: 10.3390/nu14122387

[3]. Jayaraman, S., Natarajan, S. R., Ponnusamy, B., Veeraraghavan, V. P. and Jasmine, S., 2023. Unlocking the potential of beta sitosterol: Augmenting the suppression of oral cancer cells through extrinsic and intrinsic signalling mechanisms. *The Saudi Dental Journal*, 35(8), pp.1007-1013.

[4]. Jayaraman, R., Subramani, S., Sheik Abdullah S. H., & Udaiyar M., 2018, Antihyperglycemic effect of hesperidin, a citrus flavonoid, extenuates hyperglycemia and exploring the potential role in antioxidant and antihyperlipidemic in streptozotocin-induced diabetic rats. *Biomedicine and Pharmacotherapy*, 97, 98-106. doi: 10.1016/j.biopha.2017.10.102.

[5]. Al-Ishaq, R. K., Abotaleb M., Kubatka P., Kajo K., & Büsselberg D., 2019, Flavonoids and Their Anti-Diabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. *Biomolecules*, 9(9), 430. doi:10.3390/biom9090430.

[6]. Sundaram, R., Nandhakumar E., & Haseena B. H., 2019, Hesperidin, a citrus flavonoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats, *Toxicology Mechanisms and Methods*, 29:9, 644-653.

[7]. de Souza P, da Silva R. C. V., Mariano, L. N. B., Dick, S. L., & Ventura GC, Cechinel-Filho V, 2022, Diuretic and Natriuretic Effects of Hesperidin, a Flavanone Glycoside, in

Female and Male Hypertensive Rats. *Plants (Basel*), 21, 12(1):25.
[8]. Sruthi, M. A., Mani, G., Ramakrishnan, M. and Selvaraj, J., 2023, Dental caries as a source of Helicobacter pylori infection in children: An RT-PCR study. *International Journal of Paediatric Dentistry*, 33(1), pp.82-88.
[9]. Peng, P., Jin J., Zou G., Sui Y., Han Y, Zhao D., & Liu L., 2021. Hesperidin prevents hyperglycemia in diabetic rats by activating the insulin receptor pathway. *Experimental and Therapeutic Medicine*, 21(1):53.
[10]. Mahmoud, A. M., Ahmed, O. M., Ashour, M. B., et al., 2015, *In vivo* and *in vitro* antidiabetic effects of citrus flavonoids; a study on the mechanism of action. *International Journal of Diabetes in Developing Countries*, 35(3), 250-263.
[11]. Krishnan, R. P., Pandiar, D., Ramani, P. and Jayaraman, S., 2025, Molecular profiling of oral epithelial dysplasia and oral squamous cell carcinoma using next generation sequencing. *Journal of Stomatology, Oral and Maxillofacial Surgery*, 126(4), p.102120.
[12]. Mahmoud, A. M., 2016, Hesperidin as a Promising Anti-Diabetic Flavonoid: the Underlying Molecular Mechanism. *Int Journal of Food and Nutritional Science*, 3, 313-314.
[13]. Holliday, R., 2006, Epigenetics: a historical overview. *Epigenetics*, 1: 76– 80.
[14]. Weinhold. B., 2006, Epigenetics: the science of change. *Environ Health Perspect*, 114(3), A160-7.
[15]. Berger, S. L., Kouzarides, T., Shiekhattar, R., Shilatifard, A., 2009, An operational definition of epigenetics. *Genes & Development*, 23(7), 781-3. doi: 10.1101/gad.1787609.
[16]. Jayaraman, S., Natarajan, S. R., Veeraraghavan, V. P. and Jasmine, S., 2023, Unveiling the anti-cancer mechanisms of calotropin: Insights into cell growth inhibition, cell cycle arrest, and metabolic regulation in human oral squamous carcinoma cells (HSC-3). *Journal of Oral Biology and Craniofacial Research*, 13(6), pp.704-713.
[17]. Moosavi, A., Motevalizadeh A. A., 2016, Role of Epigenetics in Biology and Human Diseases. *Iranian Biomedical Journal*, 20(5), 246-58. doi: 10.22045/ibj.2016.01.
[18]. Kawada, J., 1992, New hypotheses for the mechanisms of streptozotocin and alloxan inducing Diabetes mellitus. *Yakugaku Zasshi*, 112(11), 773-91. Japanese. doi: 10.1248/ yakushi1947. 112.11_773
[19]. Szkudelski, T., 2001, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, 50(6):537-46.
[20]. Chen, S., Gan, D., Lin, S., Zhong, Y., Chen M., Zou X., Shao Z., & Xiao, G., 2022, Metformin in aging and aging-related diseases: clinical applications and relevant mechanisms. *Theranostics*, 12(6), 2722-2740. doi: 10.7150/thno.71360.
[21]. Gurina, T. S., Simms, L., Histology, Staining. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books /NBK557663/
[22]. Tahir, R. A., Zheng, D. A., Nazir, A., & Qing, H., 2019, A review of computational algorithms for CpG islands detection. *Journal of Biosciences*, 44(6), 143.
[23]. Patrick, P. L., Lam., Mitsuyo O., Subhankar D., Yu H., Tairan Q., Tao L., Dan Z., Youhou, K., Yunfeng, L., Maria, K., Li X., Wilson C. Y., et al., 2013, Munc18b Is a Major Mediator of Insulin Exocytosis in Rat Pancreatic β-Cells. *Diabetes*, 62(7), 2416–2428. https://doi.org/10.2337/db12-1380
[24]. Świderska E., Strycharz, J., Wróblewski, A., Szemraj J., Drzewoski J., & Śliwińska A., 2020, Role of PI3K/AKT Pathway in Insulin-Mediated Glucose Uptake [Internet]. *Blood Glucose Levels*. IntechOpen; 2020. http://dx.doi.org/10.5772/intechopen.80402
[25].Fathima, J. S., Jayaraman, S., Sekar, R. and Syed, N. H., 2024, The role of

MicroRNAs in the diagnosis and treatment of oral premalignant disorders. *Odontology, pp.1-10.*

[26]. Ivorra, M. D., Paya, M., Villar, A., 1989, A review of natural products and plants as potential antidiabetic drugs. *Journal of Ethnopharmacology*, 27(3), 243–275.

[27]. Anil, B., 2010, Gaikwad, Jeena Gupta, Kulbhushan Tikoo; Epigenetic changes and alteration of Fbn1 and Col3A1 gene expression under hyperglycaemic and hyperinsulinaemic conditions. *Biochemistry Journal*, 432 (2), 333–341. doi: https://doi.org/10.1042/BJ20100414

[28]. Yasothkumar, D., Ramani, P., Jayaraman, S., Ramalingam, K. and Tilakaratne, W. M., 2024, Expression Profile of Circulating Exosomal microRNAs in Leukoplakia, Oral Submucous Fibrosis, and Combined Lesions of Leukoplakia and Oral Submucous Fibrosis. *Head and Neck Pathology*, 18(1), p.28.

[29]. Bansal, A., & Pinney, S. E., 2017, DNA methylation and its role in the pathogenesis of diabetes. *Pediatr Diabetes*, 18(3),167-177. doi: 10.1111/pedi.12521.

[30]. Sagar, S., Ramani, P., Moses, S., Gheena, S. and Selvaraj, J., 2024, Correlation of salivary cytokine IL-17A and 1, 25 dihydroxycholecalciferol in patients undergoing orthodontic treatment. Odontology, pp.1-10.

[31]. Thomas, D. D., Corkey, B. E., Istfan N.W., & Apovian C. M., 2019, Hyperinsulinemia: An Early Indicator of Metabolic Dysfunction. *Journal of the Endocrine Society*, 3(9),1727-1747. doi: 10.1210/js.2019-00065.

[32]. Wilcox. G., 2005, Insulin and insulin resistance. Clinical Biochemist Reviews, 2005 26(2), 19-39.

[33]. Zhao, C., Yang C., Wai, S. T. C., Zhang Y. P., Portillo M, Paoli P., Wu Y., San Cheang W., Liu B., Carpéné C., Xiao J., & Cao H., 2019, Regulation of glucose metabolism by bioactive phytochemicals for the management of type 2 diabetes mellitus. *Critical Reviews in Food Science and Nutrition*; 59(6):830-847. doi: 10.1080/10408398.2018.1501658.

[34]. Stanley M. P. P., & Kamalakkannan N., 2006, Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. *Journal of Biochemical and Molecular Toxicology*, 20(2), 96-102. doi: 10.1002/jbt.20117.

[35]. Latha M, & Pari L. 2003, Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. *Clinical and Experimental Pharmacology and Physiology*, 30(1-2):38-43.

[36]. Jayachandran, M., Zhang, T., Ganesan, K., Xu, B., Chung, & S.S.M. .2018, Isoquercetin ameliorates hyperglycemia and regulates key enzymes of glucose metabolism via insulin signaling pathway in streptozotocin-induced diabetic rats. *European Journal of Pharmacology*, 82:112–120.

[37]. Pari, L, & Rajarajeswari, N., 2009. Efficacy of coumarin on hepatic key enzymes of glucose metabolism in chemical induced type 2 diabetic rats. *Chemico-Biological Interactions*, 181:292–296.

[38]. Gothandam, K., Ganesan, V. S., Ayyasamy, T., & Ramalingam, S., 2019, Antioxidant potential of theaflavin ameliorates the activities of key enzymes of glucose metabolism in high fat diet and streptozotocin–induced diabetic rats. *Redox Report*, 24:41–50.

[39]. Tian, M., Han, Y. B., Zhao, C. C., et al. 2021, Hesperidin alleviates insulin resistance by improving HG-induced oxidative stress and mitochondrial dysfunction by restoring miR-149. Diabetol Metab Syndr 13:50. https://doi.org/10.1186/s13098-021-00664-1.

[40]. Chen C, Peng S, Chen F, Liu L, Li Z, Zeng G, Huang Q. 2017, Protective effects of pioglitazone on vascular endothelial cell dysfunction induced by high glucose via

inhibition of IKKα/β-NFκB signaling mediated by PPARγ *in vitro*. *Canadian Journal of Physiology and Pharmacology*, 95(12), 1480-1487. doi: 10.1139/cjpp-2016-0574.

[41]. Copps, K.D.;,White, M.F. 2012, Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia*, 55:2565–2582.

[42]. Chuang, W.T., Yen, C.C., Huang, C.S., Chen, H.W., Lii, C. K**.,** 2020, Benzyl Isothiocyanate Ameliorates High-Fat Diet-Induced Hyperglycemia by Enhancing Nrf2-Dependent Antioxidant Defense-Mediated IRS-1/AKT/TBC1D1 Signaling and GLUT4 Expression in Skeletal Muscle. *Journal of Agricultural and Food Chemistry*, 2020, 68, 15228–15238.

[43]. Boucher, J., Kleinridders, A., Kahn, C. R., 2014, Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harbor Perspectives in Biology, 2014, 6, a009191.

[44]. Zhang, Y. Xu, W., Huang, X., Zhao, Y., Ren, Q., Hong, Z., Huang, M., Xing, X., 2018, Fucoxanthin ameliorates hyperglycemia, hyperlipidemia and insulin resistance in diabetic rats partially through IRS-1/PI3K/Akt and AMPK pathways. *Journal of Functional Foods*, 48, 515–524.

[45]. Cai, S., Sun, W., Fan, Y., Guo, X., Xu, G., Xu, T., Hou, Y., Zhao, B., Feng, & X., Liu, 2016. T. Effect of mulberry leaf (*Folium Mori*) on insulin resistance via IRS-1/PI3K/Glut-4 signalling pathway in type 2 diabetes mellitus rats. Pharmaceutical Biology, 54, 2685–2691.

[46]. Hsu, W. H., Hsiao, P. J., Lin, P. C., Chen, S. C., Lee, M. Y., Shin, S. J., 2017. Effect of metformin on kidney function in patients with type 2 diabetes mellitus and moderate chronic kidney disease. *Oncotarget*, 9(4), 5416-5423. doi: 10.18632/oncotarget.23387.

[47]. Bandeira Sde, M, Guedes Gda, S, da Fonseca, L. J., Pires, A. S., Gelain, D. P., Moreira J. C., Rabelo, L. A., Vasconcelos, S. M., Goulart, M. O., 2012, Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. *Oxid Med Cell Longev*, 819310. doi: 10.1155/2012/819310.