Texila International Journal of Public Health
Special Issue 2023
DOI: 10.21522/TIJPH.2013.SE.23.01.Art002

Sativoside Mitigates High-Fat Diet-Induced Inflammation and Type-2 Diabetes in Adipose Tissue of Wistar Rats

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Abstract

This study aimed to investigate the impact of Stevioside, on the biochemical changes in high-fat diet-fed Wistar rats. Adult male Wistar rats were induced into a diabetic state through the administration of a high-fat diet and sucrose for 60 days, followed by oral administration of stevioside (20 mg/kg/day) for 45 days. Various parameters, including fasting blood glucose, oral glucose tolerance, insulin, insulin tolerance, liver function (ALT, AST, ALP), kidney function (urea and creatinine), and lipid profiles (TC, TG, FFA, HDL-c and LDL-c), serum adipokines levels such as adiponectin, leptin, resistin were assessed. Stevioside treatment notably improved glucose and insulin tolerances in diabetic rats and normalized their elevated levels of fasting blood glucose, serum insulin, and lipid profile. In the high-fat diet-induced type 2 diabetes rat model, Stevioside effectively restored the altered blood serum levels, demonstrating efficacy comparable to that of metformin. Therefore, Stevioside displays promise as a potential phytomedicine for managing type 2 diabetes mellitus.

Keywords: *High-fat diet, Insulin tolerance, Type-2 diabetes, Stevia rebaudiana.*

Introduction

Diabetes is a metabolic disorder characterized by persistent increased levels of blood sugar, along with irregularities in blood lipids and proteins, as well as additional symptoms that elevate the risk of illness and death [1]. Diabetes poses a major challenge to public health in both the United States and globally, with its classification including type 1, type 2, and gestational diabetes [2-5]. Type 2 diabetes mellitus (T2DM) is an escalating health concern that has now reached the level of a worldwide pandemic, resulting in significant economic implications globally. Most of the global diabetes population, approximately 60%, resides in Asia, with projections indicating that this will increase further in the coming years [6].

The role of diet is pivotal in the progress of diabetes in humans. Elevated consumption of high-fat diets and the adoption of Western dietary patterns have been associated with insulin resistance and an increased susceptibility to diabetes mellitus and related metabolic disorders [7]. Currently, accessible oral hypoglycemic drugs demonstrate numerous adverse effects. Consequently, there is a demand for more potent oral antihyperglycemic agents, especially those that can restore both insulin and glucose levels to normal. A diverse range of plants and their active constituents, with minimal side effects, offer an alternative therapy for T2DM. Furthermore, the realm of plants represents a significantly underexplored source of biologically active compounds. *Stevia rebaudiana* is a plant species native to South America. It is well-known for its sweet-tasting leaves, which contain natural non-caloric sweeteners [8]. The sweet compounds in the leaves are called steviol glycosides, with stevioside and rebaudioside being the most

Received: 01.12.2023 ***Accepted: 08.12.2023*** ***Published on: 30.12.2023***
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abundant and commercially significant. Stevia has garnered attention as a natural alternative to artificial sweeteners, with a growing demand as a sugar substitute in the food and beverage industry [9]. It is several hundred times sweeter than sugar, yet it does not contribute calories or carbohydrates to the diet. This characteristic makes it a favorable option for individuals looking to reduce their calorie intake or manage conditions like obesity and diabetes. Apart from its use as a sweetener, *Stevia rebaudiana* has been explored for its potential medicinal properties [10]. Research suggests that it may have hypoglycemic [11], antihypertensive [12], anti-inflammatory [13], and antioxidant effects (Lemus-Mondaca et al., [14]. As a result, *Stevia rebaudiana* has attracted attention to the development of natural therapies for conditions such as diabetes, hypertension, and metabolic syndrome [15-17] . Therefore, the present study aims to determine the stability of the model induced by HFD feeding combined with sucrose water. Establishing the stability of the model will benefit the application of this model in future pharmacological studies.

Materials and Methods

Chemicals and Kits

The entire chemicals and reagents used in this research were of the molecular and analytical grade. Stevioside was purchased from Merk, Germany. Insulin ELISA kit was purchased from Krishgen Biosystems, Mumbai. Biochemical assay kits were procured from Spinreact, Spain, and Adipokinesultra-sensitive enzyme-linked immunosorbent assay (ELISA) kits were obtained from Ray Biotech.

Experimental Design and Diabetic Induction

Male Wistar rats weighing 170–190 g was included in this investigation. The rats were maintained under standard conditions of temperature (23°C ± 1°C) and humidity (50%–60%) on a 12-h light/dark cycle with free access to food and water. The animal study protocol was approved by the Research Ethics Committee of Saveetha Dental College, SIMATS (IAEC No: BRULAC/SDCH/SIMATS/IAEC/04-2022/107 dated 25th April 2022). The rats were randomly divided into four groups (n = 6). Group I served as the control, Group II consisted of T2DM rats, Group III comprised T2DM rats treated orally with Stevioside (20 mg/kg/day), and Group IV contained T2DM rats treated orally with metformin (50 mg/kg/day) for 45 days. Before the animals' sacrifice, all groups underwent an Oral Glucose Tolerance test (OGTT) and FBG analysis two days earlier. Following the experimental period, the animals were euthanized, and blood samples were collected and stored at −20 °C after serum separation. Other organs were promptly excised from the rats and preserved at −80 °C for subsequent analysis.

T2DM was induced in the experimental rats through the administration of HFD consisting of 66% standard rat feed, 30% coconut oil, 3% cholesterol, and 1% cholic acid over a 60-day duration. Alongside the HFD, the rats were provided with 30% sucrose in their drinking water. To confirm the development of diabetes, the rats underwent an overnight fast on the 58th day of the experiment, following which their fasting blood glucose (FBG) levels were measured. Rats with FBG levels exceeding 120 mg/dL were identified as having T2DM and were subsequently maintained on the HFD and sucrose water until the study's conclusion.

Biochemical Analysis

Effects of Stevioside on FBG and Serum Insulin

Following an overnight fasting period, blood samples were collected from the rat's tail tip, and the FBG levels were evaluated using Caresens N blood glucose test strips (ISENS Biosensors India Private Limited, Gurgaon, India), with results presented in mg/dL. A commercially accessible rat insulin ELISA kit (Krishgen Biosystems, Mumbai) was employed for the quantification of serum insulin levels. The

detection range of the kit was 0.1 to 64 ng/ml. The insulin concentration was expressed in µIU/ml.

Stevioside Effect on LFT, KFT Markers

Biochemical assay kits procured from Spinreact, Spain, were utilized to detect liver function markers, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), along with kidney function markers, namely, urea and creatinine. The outcomes were reported in units per liter (U/L).

Effects of Stevioside on Lipid Profiles

Serum triglyceride (TG), free fatty acid (FFA), total cholesterol (TC), high-density lipoproteins (HDL-c), and low-density lipoproteins (LDL-c) were analyzed using the Spinreact assay kit (Spain) according to the manufacturer's instructions. The findings were reported in milligrams per deciliter (mg/dL).

Effects of Stevioside on Adiponectin, Leptin, Resistin

Serum adiponectin, resistin, and leptin levels were measured using the rat insulin ELISA kit obtained from RayBiotech, USA. Adiponectin and resistin results were reported in nanograms per milliliter (ng/mL), while the leptin levels were expressed in picograms per milliliter (pg/mL).

Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA), and variations between the mean values were determined using Duncan's multiple range test with GraphPad Prism version 8. Results were considered statistically significant at the $p < 0.05$ level.

Results

Biochemical Analysis

Effects of Stevioside on FBG, and Serum Insulin

To assess the potential anti-diabetic effects of Stevioside, FBG was conducted in the control and experimental animal groups. As depicted in Figure 1, the administration of Stevioside exhibited a notable reduction in elevated FBG levels in diabetic rats, comparable to the effects of metformin.

Figure 1 illustrates the serum insulin levels in the various groups of rats included in the study. Nevertheless, the Stevioside administration group showed near the normal range, thereby demonstrating its potential to enhance insulin sensitivity.

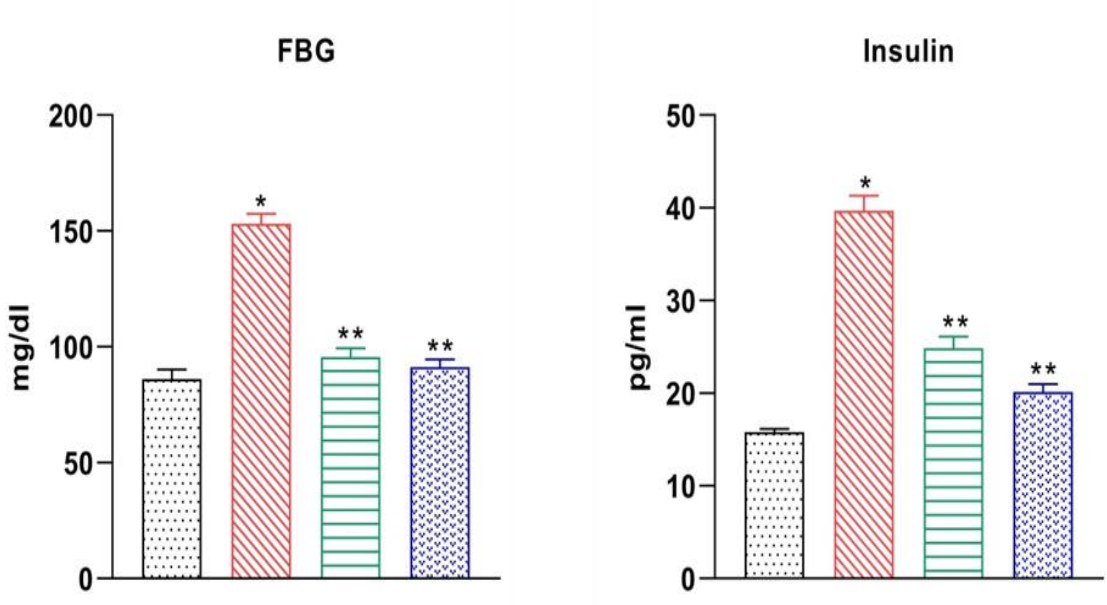

Figure 1. Effect of Stevioside on FBG, Insulin in Type-2 Diabetic Rats

Stevioside Effect on LFT, KFT

In the diabetic group of animals, liver function markers such as ALT, AST, and ALP (Figure 2), along with renal function markers like urea and creatinine (Figure 2), were found to be elevated. Additionally, treatment with Stevioside effectively reduced these markers, demonstrating efficacy like the standard drug such as metformin.

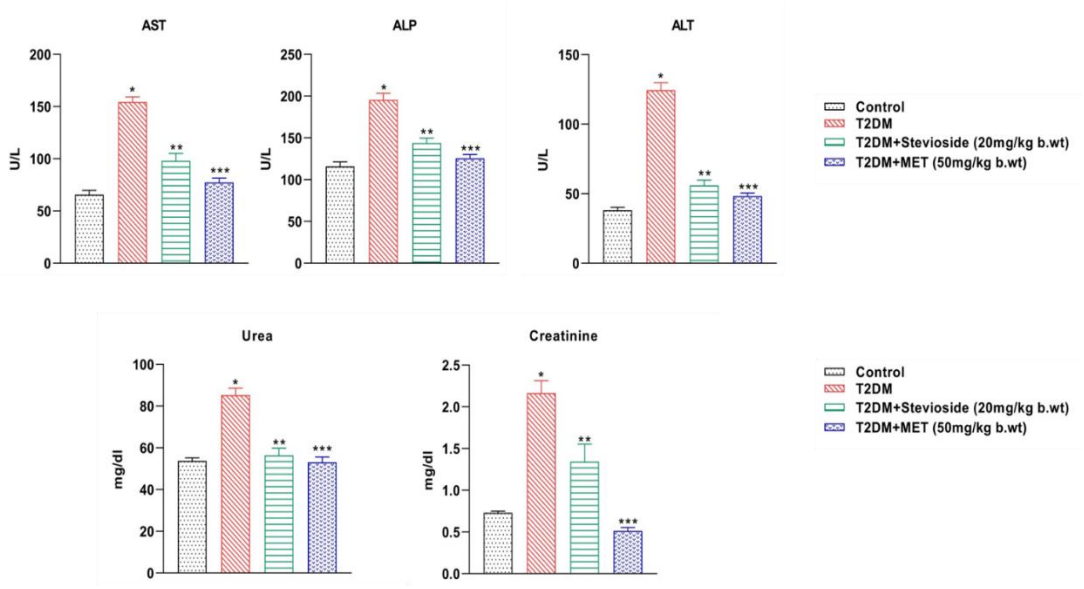

Figure 2. Effect of Stevioside on Liver Function Markers (AST, ALP, and ALT) and Kidney Function such as Urea and Creatinine in Type-2 Diabetic Rats

Effects of Stevioside on Lipid Profiles

To assess the potential hypolipidemic effects of Stevioside, the serum levels of TC, TG, FFA, HDL-c, and LDL-c were measured in different treatment groups (Figure 3). Diabetic rats displayed signs of dyslipidemia, evidenced by a considerable increase in their serum TC, TG, FFA, and LDL-c levels, along with decreased HDL levels when compared to the control groups. However, Stevioside effectively ameliorated dyslipidemia in diabetic rats, akin to the effects of metformin, by restoring these lipid profile values to normal levels, as depicted in Figure 3.

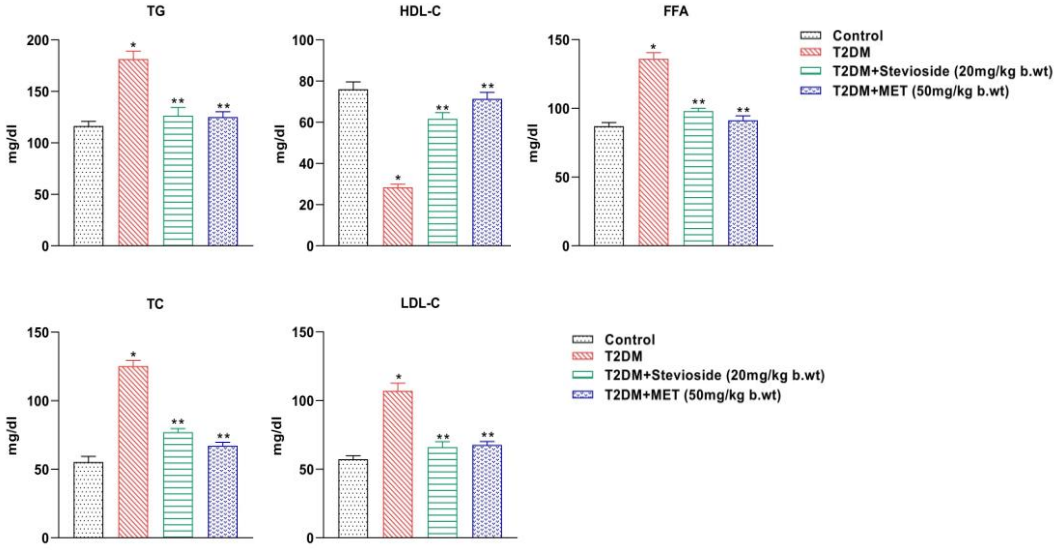

Figure 3. Effect of Stevioside on Lipid Profiles such as TC, TG, FFA, HDL-c, and LDL-c

Effects of Stevioside on Adiponectin, Leptin, Resistin

Adiponectin, leptin, and resistin are recognized as potential serum markers of metabolic syndrome. Therefore, their levels were evaluated in the serum of both the control and experimental groups of rats. The findings indicated a notable increase ($p < 0.05$) in leptin and resistin concentrations, coupled with a significant decrease in adiponectin levels in diabetic rats. However, treatment with Stevioside effectively normalized the dysregulated adipokine levels in type-2 diabetic rats, showing comparable efficacy to that of metformin (Figure 4).

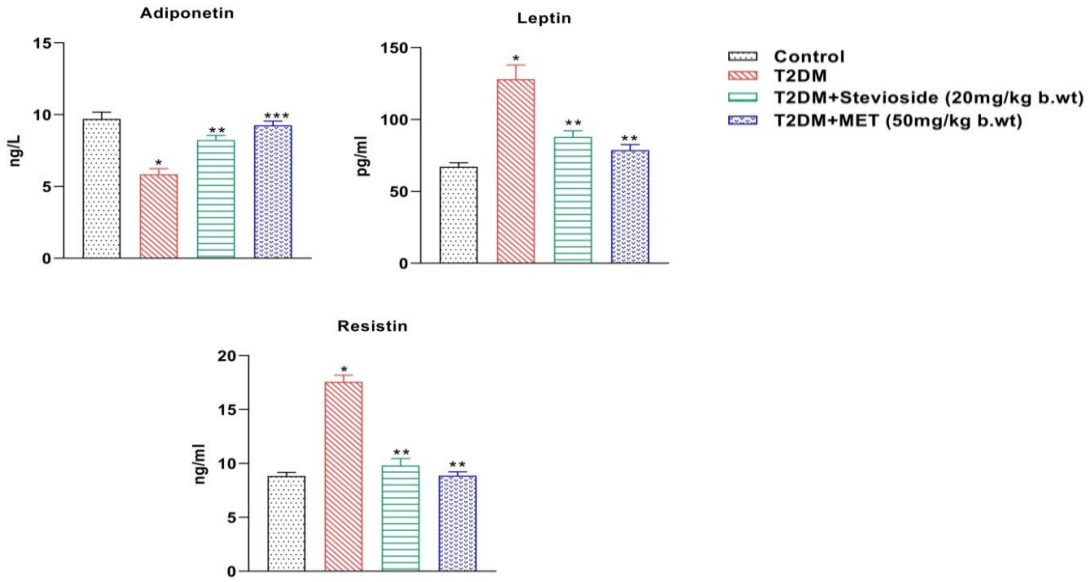

Figure 4. Effect of Stevioside on Lipid Profiles such as TC, TG, FFA, HDL-c, and LDL-c. Discussion

T2DM is a multifaceted, varied, and polygenic condition characterized by reduced insulin function (insulin resistance), along with the failure of β-cells to produce adequate insulin to counteract the insulin resistance (pancreatic β-cell dysfunction) [18]. Animal models need to emulate the phenotype and reproduce the disease's developmental process to apply to human conditions. Following 8 weeks of HFD feeding all the rats exhibited abdominal obesity, dyslipidemia, hyperglycemia, and insulin resistance. effectively replicating the natural progression of the early stages of T2DM. This serves as a crucial foundation for establishing the T2DM model [19-21].

In this study, the development of obesity by HFD in group II rats is evident by the profound increase in the FBG level. However, stevioside treatment in rats fed with HFD significantly limits the FBG level which in turn suggests that stevioside could act as a potent antiobesity drug. More importantly, after the glucose load, the blood glucose level of type 2 diabetic rats gradually increased and reached its peak value at 1 h. The elevated glucose level did not reach the normal value of 120 mg/dL even after 2 h of glucose administration, which in turn indicates glucose tolerance in these diabetic rats. However, stevioside-treated diabetic rats exhibit improved glucose tolerance as effectively as those treated with metformin. Control rats did not display any variation in glucose levels during ITT [22,23]. Likewise, insulin treatment slowly decreased the blood sugar level in type 2 diabetic rats with the minimal level being achieved only after 1 h. However, stevioside increased insulin tolerance in diabetic rats very effectively like metformin. Taken together, this evidence proves the insulin-sensitizing potential of stevioside in T2DM rats [24-26].

Stevioside effectively scavenged the surplus reactive oxygen species (ROS) generated by the

HFD, enhanced the antioxidants, and reinstated liver function in the rat model of insulin resistance caused by high fat intake. Consequently, our study findings demonstrate that Stevioside could successfully counteract oxidative stress in the gastrocnemius muscles of rats with T2DM. This study aimed to explore whether Stevioside could reverse the oxidative stress-induced reduction of insulin signaling. As anticipated, Group II rats fed with HFD and sucrose exhibited a notable increase in serum lipid profile, including FFA, TG, TC, and LDL-c, alongside a significant decrease in HDL cholesterol levels. These findings highlight the development of obesity and dyslipidemia in diabetic group rats due to excessive fat consumption [27-29]. Nonetheless, the administration of Stevioside to these diabetic rats notably improved the serum lipid profile, concurrently leading to an increase in HDL cholesterol levels, indicative of its hypocholesterolemic properties. Previous studies have reported that the application of various doses of Stevioside to diabetic rats effectively alleviated dyslipidemia [28, 30].

Insulin resistance in obese individuals primarily stems from comprehensive alterations in the metabolic and inflammatory functions of adipocytes. In the context of obesity, adipocytes tend to accumulate higher lipid levels and secrete elevated amounts of proinflammatory adipokines (such as leptin and resistin), while displaying reduced levels of anti-inflammatory adipokines (like adiponectin). These lipids and proinflammatory adipokines activate the IKKβ/NF-κB and JNK signaling pathways, subsequently stimulating the production of proinflammatory cytokines, namely TNF-α and IL-6 [31]. These cytokines facilitate the phosphorylation of serine kinases of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2), ultimately obstructing insulin signaling and glucose uptake. This represents the primary molecular and signaling mechanism underlying obesity-induced insulin resistance [20,21,32].

In the HFD and sucrose-induced type 2 diabetic rats, there was a notable elevation in serum leptin and resistin levels, alongside a significant decrease in serum adiponectin levels when compared to the control rats. Leptin, resistin, and adiponectin are crucial adipocytokines secreted by adipose tissues to communicate with various organs, including the brain, pancreas, muscle, and liver [33-36]. The current study has several limitations. Prolonged monitoring of the model and the assessment of biochemical parameters at various time points could offer additional insights, including identifying the period during which fasting blood glucose (FBG) stabilizes. Furthermore, further investigation is warranted to elucidate the underlying mechanisms contributing to the recovery observed in the model.

Conclusion

Our current results unequivocally demonstrate the ameliorative effects of Stevioside in the high-fat diet-induced rat model. Therefore, our study concludes that the inclusion of Stevioside as a supplement could offer a beneficial strategy for the control of T2DM. Further investigations focusing on elucidating the role of Stevioside in cell line models are necessary to establish its mechanism of action, paving the way for potential clinical trials involving this natural compound for the treatment of T2DM.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors extend their sincere appreciation to Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences in Chennai, for their steadfast support, which played a crucial role in the successful completion of this study.

References

[1] Farag YM, Gaballa MR. Diabesity: an overview of a rising epidemic. *Nephrol Dial Transplant*. 2011 Jan;26(1):28-35. doi: 10.1093/ndt/gfq576. Epub 2010 Nov 2. PMID: 21045078.

[2] Xu G, Liu B, Sun Y, Du Y, Snetselaar LG, Hu FB, Bao W. Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. *BMJ. 2018* Sep 4;362:k1497. doi: 10.1136/bmj.k1497. PMID: 30181166; PMCID: PMC6122253.

[3] Prasad M, Rajagopal P, Devarajan N, Veeraraghavan VP, Palanisamy CP, Cui B, Patil S, Jayaraman S. A comprehensive review on high -fat diet-induced diabetes mellitus: an epigenetic view. *J Nutr Biochem.* 2022 Sep;107:109037. doi: 10.1016/j.jnutbio.2022.109037. Epub 2022 May 6. PMID: 35533900.

[4] Prasad M, Jayaraman S, Natarajan SR, Veeraraghavan VP, Krishnamoorthy R, Gatasheh MK, Palanisamy CP, Elrobh M. Piperine modulates IR/Akt/GLUT4 pathways to mitigate insulin resistance: Evidence from animal and computational studies. *Int J Biol Macromol.* 2023 Dec 31;253(Pt 5):127242. doi: 10.1016/j.ijbiomac.2023.127242. Epub 2023 Oct 4. PMID: 37797864.

[5] Kiruthigha T, Gayathri R, Vishnu Priya V, Selvaraj J, Kavitha, S. Piperine Modulates High Fat Diet - Induced Renal Damage by Regulating Kim-1 and Igf-1 Beta Signaling Molecules in Male Wistar Rats". *J. Adv. Zool.* 2023 44 (S5):246-54.

[6] Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, Nichols G. Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010 Mar;87(3):293-301. doi: 10.1016/j.diabres.2010.01.026. Epub 2010 Feb 19. Erratum in: Diabetes Res Clin Pract. 2011 May;92(2):301. PMID: 20171754.

[7] Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, Jeandidier N, Maillard E, Marchioni E, Sigrist S, Dal S. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. Nutr Metab (Lond). 2016 Feb 25;13:15. doi: 10.1186/s12986-016-0074-1. PMID: 26918024; PMCID: PMC4766713.

[8] Goyal SK, Samsher, Goyal RK. Stevia (Stevia rebaudiana) a bio-sweetener: a review. *Int J Food Sci Nutr.* 2010 Feb;61(1):1-10. doi: 10.3109/09637480903193049. PMID: 19961353.

[9] Samuel P, Ayoob KT, Magnuson BA, Wölwer-Rieck U, Jeppesen PB, Rogers PJ, Rowland I, Mathews R. Stevia Leaf to Stevia Sweetener: Exploring Its Science, Benefits, and Future Potential. *J Nutr. 2018* Jul 1;148(7):1186S-1205S. doi: 10.1093/jn/nxy102. PMID: 29982648.

[10] Orellana-Paucar AM. Steviol Glycosides from Stevia rebaudiana: An Updated Overview of Their Sweetening Activity, Pharmacological Properties, and Safety Aspects. Molecules. 2023 Jan 27;28(3):1258. doi: 10.3390/molecules28031258. PMID: 36770924; PMCID: PMC9920402.

[11] Barriocanal LA, Palacios M, Benitez G, Benitez S, Jimenez JT, Jimenez N, Rojas V. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. *Regul Toxicol Pharmacol.* 2008 Jun;51(1):37-41. doi: 10.1016/j.yrtph.2008.02.006. Epub 2008 Mar 5. PMID: 18397817.

[12] Carrera-Lanestosa A, Moguel-Ordóñez Y, Segura-Campos M. Stevia rebaudiana Bertoni: A Natural Alternative for Treating Diseases Associated with Metabolic Syndrome. *J Med Food*. 2017 Oct;20(10):933-943. doi: 10.1089/jmf.2016.0171. Epub 2017 Aug 9. PMID: 28792778; PMCID: PMC5651958.

[13] Ruiz-Ruiz JC, Moguel-Ordoñez YB, Segura-Campos MR. Biological activity of Stevia rebaudiana Bertoni and their relationship to health. *Crit Rev Food Sci Nutr.* 2017 Aug 13;57(12):2680-2690. doi: 10.1080/10408398.2015.1072083. PMID: 26479769.

[14] Lemus-Mondaca R, Vega-Gálvez A, Zura-Bravo L, Ah-Hen K. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional, and functional aspects. Food Chem. 2012 Jun 1;132(3):1121-1132. doi: 10.1016/j.foodchem.2011.11.140. Epub 2011 Dec 13. PMID: 29243591.

[15] Thana Lakshme, P.S., Gayathri, R., Vishnu Priya V. Preliminary Phytochemical Screening and Estimation of Total Phenolic Content of Aqueous Cladode Extract of Opuntia dilleniid. *J. Res. Med. Dent. Sci.* 2021 9(2): 254-257.

[16] Mithil Vora, Vishnu Priya V, Selvaraj J, Gayathri R, Kavitha S. Effect of Lupeol on proinflammatory Markers in Adipose Tissue of High-Fat Diet and Sucrose Induced Type-2 Diabetic Rats. *J. Res. Med. Dent. Sci.* 2021 9(10):116-121.

[17] Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. *J Adv Pharm Technol Res*. 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr_189_22. Epub 2022 Dec 30. PMID: 36798576; PMCID: PMC9926592.

[18] Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *J Diabetes Investig.* 2014 Jul;5(4):349-58. doi: 10.1111/jdi.12235. Epub 2014 May 19. PMID: 25411593; PMCID: PMC4210077.

[19] Holmes A, Coppey LJ, Davidson EP, Yorek MA. Rat Models of Diet-Induced Obesity and High Fat/Low Dose Streptozotocin Type 2 Diabetes: Effect of Reversal of High Fat Diet Compared to Treatment with Enalapril or Menhaden Oil on Glucose Utilization and Neuropathic Endpoints. *J Diabetes Res.* 2015;2015:307285. doi: 10.1155/2015/307285. Epub 2015 Jul 2. PMID: 26229968.

[20] Dev Arora, Gayathri R, Selvaraj J, Vishnu Priya V, Kavitha S. Vitamin C and E Down Regulates the Expression of C-JNK, IKKB, NF-kB in Adipose Tissue of PCB-Exposed Rats. *J. Res. Med. Dent. Sci.* 2021 9(11):39-44.

[21] Khan, HLA, Sridevi G, Selvaraj J, Preetha S. In vitro Anti-inflammatory Properties in Various Extracts (Ethanol, Chloroform and Aqueous) of Kaempferia galanga Linn Rhizome. *J. Pharm. Res. Int.* 2021 33 (47B): 476–481. DOI:https://doi.org/10.9734/jpri/2021/v33i47B33146.

[22] McGuinness OP, Ayala JE, Laughlin MR, Wasserman DH. NIH experiment in centralized mouse phenotyping: the Vanderbilt experience and recommendations for evaluating glucose homeostasis in the mouse. *Am J Physiol Endocrinol Metab.* 2009 Oct;297(4):E849-55. doi: 10.1152/ajpendo.90996.2008. Epub 2009 Jul 28. PMID: 19638507; PMCID: PMC2763792.

[23] Nagy C, Einwallner E. Study of In Vivo Glucose Metabolism in High-fat Diet-fed Mice Using Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT). *J Vis Ex*p. 2018 Jan 7;(131):56672. doi: 10.3791/56672. PMID: 29364280; PMCID: PMC5908452.

[24] Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab*. 2008 Dec;295(6):E1323-32. doi: 10.1152/ajpendo.90617.2008. Epub 2008 Sep 23. PMID: 18812462.

[25] Akifa Begum, Palati Sinduja, Priyadharshini R, Selvaraj Jayaraman. Estimation of Clinocopathological Correlation and Comparison of Salivary TNF-α among Normal and Post Radiotherapy Patients of Oral cancer-A Cross-Sectional Study. *J. Res. Med. Dent*. Sci. 2021 9(10): 92-97.

[26] Fathima Hinaz Z, Gayathri R, Selvaraj J, Vishnu Priya V, Kavitha, S, Gayathri R. Comparative Evaluation of Anti-Cholesterol Potential of Apple Cider Vinegar and Its Herbal Formulation with Allium Sativum and Honey-An In-Vitro Assay. *J. Res. Med. Dent. Sci.* 2021 9 (10),142-147.

[27] Logan IE, Bobe G, Miranda CL, Vasquez-Perez S, Choi J, Lowry MB, Sharpton TJ, Morgun A, Maier CS, Stevens JF, Shulzhenko N, Gombart AF. Germ-Free Swiss Webster Mice on a High-Fat Diet Develop Obesity, Hyperglycemia, and Dyslipidemia. Microorganisms. 2020 Apr 5;8(4):520. doi: 10.3390/microorganisms8040520. PMID: 32260528; PMCID: PMC7232377.

[28] Rotimi SO, Rotimi OA, Adelani IB, Onuzulu C, Obi P, Okungbaye R. Stevioside modulates oxidative damage in the liver and kidney of high fat/low streptozocin diabetic rats. Heliyon. 2018 May 31;4(5):e00640. doi: 10.1016/j.heliyon.2018.e00640. PMID: 29872771; PMCID: PMC5986550.

[29] Mounithaa N, Gayathri R, Selvaraj Jayaraman, Vishnu Priya V, Kavitha S. Effect of Piperine on an Nrf2/Keap 1 Signalling Mechanism in Adipose

Tissue of High Fat Diet and Sucrose-Induced Experimental Diabetic Rats. *J. Adv. Zool*. 2023 44 (S5):232-39.

[30] Zhao RZ, Jiang S, Zhang L, Yu ZB. Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *Int J Mol Med.* 2019 Jul;44(1):3-15. doi: 10.3892/ijmm.2019.4188. Epub 2019 May 8. PMID: 31115493; PMCID: PMC6559295.

[31] Padmapriya, A., Preetha, S., Selvaraj, J., Sridevi, G. (2022). Effect of Carica papaya seed extract on IL-6 and TNF-α in human lung cancer cell lines-an In vitro study. *Res J Pharm Technol.* 2022 15 (12): 5478-5482.

[32] Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci. 2010 Nov;1212:E1-E19. doi: 10.1111/j.1749-6632.2010.05875.x. Erratum in: *Ann N Y Acad Sci*. 2011 May;1226(1):50. PMID: 21276002; PMCID: PMC3075414.

[33] Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Front Endocrinol (Lausanne). 2013 Jun 12;4:71. doi: 10.3389/fendo.2013.00071. PMID: 23781214; PMCID: PMC3679475.

[34] Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci.* 2014 Apr 11;15(4):6184-223. doi: 10.3390/ijms15046184. PMID: 24733068; PMCID: PMC4013623.

[35] Deenadayalan A, Subramanian V, Paramasivan V, Veeraraghavan VP, Rengasamy G, Coiambatore Sadagopan J, Rajagopal P, Jayaraman S. Stevioside Attenuates Insulin Resistance in Skeletal Muscle by Facilitating IR/IRS-1/Akt/GLUT 4 Signaling Pathways: An In Vivo and In Silico Approach. Molecules. 2021 Dec 20;26(24):7689. doi: 10.3390/molecules26247689. PMID: 34946771; PMCID: PMC8707280.

[36] Jayaraman S, Krishnamoorthy K, Prasad M, Veeraraghavan VP, Krishnamoorthy R, Alshuniaber MA, Gatasheh MK, Elrobh M, Gunassekaran. Glyphosate potentiates insulin resistance in skeletal muscle through the modulation of IRS-1/PI3K/Akt mediated mechanisms: An in vivo and in silico analysis. *Int J Biol Macromol*. 2023 Jul 1;242(Pt 2):124917. doi: 10.1016/j.ijbiomac.2023.124917. Epub 2023 May 18. PMID: 37207753.