Journal of Advanced Zoology
ISSN: 0253-7214
Volume 44 Issue S-5 Year 2023 Page 232:239

Effect of Piperine on an Nrf2/Keap 1 Signalling Mechanism in Adipose Tissue of High Fat Diet and Sucrose-Induced Experimental Diabetic Rats

Mounithaa N[1], R. Gayathri[2], Selvaraj Jayaraman[3], V. Vishnu Priya[4], Kavitha.S[5]

[1]*Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, Email: 152101005.sdc@saveetha.com*
[2]*Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, Email: gayathri.sdc@saveetha.com*
[3]*Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, jayalakshmisomasundaram@saveetha.com*
[4]*Department of Biochemistry, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India,E-mail: vishnupriya@saveetha.com*
[5]*Department of Biochemistry, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, E-mail: kavithas.sdc@saveetha.com*

**Corresponding author: R. Gayathri*

***** *Email: gayathri.sdc@saveetha.com*

Article History

Received: 23 June 2023
Revised: 09 Sept 2023
Accepted: 09 Oct 2023

Abstract

Piperine, an alkaloid compound found in black pepper has been shown to have various health benefits such as anti-oxidants, anti-inflammatory, and anticancer activities. But mechanisms underlying the anti-diabetic activity are unclear. Keap1-Nrf2 is an anti-oxidant stress signal pathway and it is considered to be an intracellular defence mechanism to countered oxidative stress. The study was aimed at assessing antidiabetic activity of piperine against high fat diet and sucrose-induced (HFD) type-2 diabetic rats by regulating the expression of Nrf2/Keap 1 signalling. Healthy adult male albino rats of wistar strain were grouped in to 5. Considering healthy control (group-1), HFD-induced type-2 diabetes (group2), Diabetic rats treated with piperine (group 3), diabetic rats metformin (group 4) and control +Piperine treated rats (group 5) respectively. After 30 days of treatment, fasting blood glucose (FBG) checked and adipose tissue from control and treated groups was used to determine the role of piperine on the expression of NrF2/Keap 1 mNRA in adipose by Real Time-PCR analysis. Data were analysed by one-way ANOVA and $p<0.01$ was considered to be statistical significance among the groups. HFD-induced T2DM showed a significant increase in the levels of FBG and altered levels of Nrf-2 and Keap-1 gene expression (2 fold) compared to normal control animals. Piperine at a dose of 40mg, fascinatingly improved the glycemic control and normalised the mRNA expression of both Nrf-2 and Keap-1 whose effects were near to that of standard drug metformin level ($p<0.05$) proving its potential mechanism of action. ***Conclusion:*** *Our current study clearly indicates that piperine controls hyperglycemia in type-2 diabetic rats by facilitating the expression of antioxidant signalling (Nrf-2/Keap-1) in the adipose. In addition, this is the first of its kind to show the role of piperine in reducing hyperglycemia against high fat diet and sucrose –induced type-2 diabetic rats as an invivo experimental model. Hence, piperine could be*

considered as an important health supplement and potential drug candidate for the treatment of diabetes.

Keywords: *High fat and sucrose diet, type-2 diabetes, Piperine, antioxidant signalling, novel method, health and well-being.*

1. Introduction

Diabetes mellitus (DM) is considered to be one of the most problematic public health issues, and its worldwide prevalence has more than doubled over the past 30 years. It is the major causes of death worldwide in particular; the incidence of DM is increasing more rapidly in Asian countries such as India, Korea, and China (Tabish, 2007). Type 2 Diabetes Mellitus (T2DM) is the most prevalent form of DM, which accounts for roughly 90% of all cases (Prasad et al., 2022). Over the decades, over nutrition coupled with a sedentary lifestyle has led to a striking increase in metabolic diseases, such as type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD). Some organs and tissues (e.g., adipose, liver, and muscle) secrete specific cytokines for inter-organ communication, and the production and secretion of these cytokines alter during nutritional stress and physical activity leads to the development of inflammation mediated development of insulin resistance or type-2 diabetes. Multicellular organisms comprise a controllable collection of cells. In general, different communication signals regulate various life activities and behaviours (Sanjay Varshan et al., 2023).

Nrf2 (Nuclear factor erythroid 2-related factor 2) is a critical transcription factor that plays a central role in cellular defense against oxidative stress and inflammation. Activation of the Nrf2 signaling pathway has garnered attention as a potential therapeutic target for diabetes management due to its ability to counteract oxidative stress and inflammation, which are implicated in the pathogenesis of diabetes. Natural compounds, such as phytochemicals found in various plants, have been studied for their ability to activate the Nrf2 pathway and potentially aid in diabetes management (Nguyen et al., 2009). When exposed to oxidative or electrophilic stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it binds to antioxidant response elements (AREs) and activates the transcription of genes encoding various antioxidant and detoxification enzymes. Dysregulation of Nrf2 signaling is implicated in the development and progression of diabetes due to increased oxidative stress and inflammation (Giacco and Brownlee, 2010).

Despite recent developments in the knowledge and treatment of diabetes mellitus, the disease's incidence is steadily rising, having negative effects on the health and longevity of people all over the world. The need for new treatment molecules has been driven by the drawbacks and adverse effects of present antidiabetic medications. The multi-pathogenicity of diabetes mellitus has led to the hypothesis that pharmacological activities “hold the key” to the development of a less expensive, more effective and safe therapeutic agent for the management of the condition and its complications. Various plants and plants compounds have been proved to be antidiabetic drugs (Vishaka et al., 2022).

Piperine is a bioactive compound found in black pepper (*Piper nigrum*) and long pepper (Piper longum). It has been studied for its potential pharmacological effects, including its impact on various aspects of health, including its potential antidiabetic properties. In vitro studies have explored the effect of piperine on different parameters related to diabetes, such as glucose uptake, insulin sensitivity, and pancreatic beta-cell function (Ampawong et al., 2016). Piperine has been reported to enhance insulin sensitivity by modulating various signaling pathways, including the AMP-activated protein kinase (AMPK) pathway and the peroxisome proliferator-activated receptor-gamma (PPARγ) pathway. It has been demonstrated protective effects on pancreatic beta cells, which are responsible for insulin production. It may help preserve beta cell function and viability in the face of oxidative stress (Maity et al., 2005; Srinivasan, 2007). However, the mechanisms underlying the possible role piperine on Nrf2/Keapt-1 signalling, an important signalling pathway involved in the protective of cells form ROS mediated development of insulin resistance has not been studied. Hence, in this study, we have provided the therapeutic effects of piperine through HFD-induced type-2 diabetic *in vivo* experimental model.

2. Materials And Methods

Chemicals

All chemicals and reagents used in this study were purchased from sigma chemical company St. Louis, MO, USA; Invitrogen, USA;

Eurofins Genomics India Pvt Ltd, Bangalore, India; New Zealand Biolabs (NEB),USA; Promega, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse transcriptase enzyme (M MULV) was purchased from New England Biolabs (NEB),USA and G0 Taq Green master mix was purchased from Promega,USA.Nrf2 Keap 1 and B actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India and MDA and GSH ELISA kits were procured from Abkkine,(Bldg CIT, Optics Valley International Biomedicine Park, Wuhan, China 430223)

Animals

Healthy adults male albino rats of Wistar strain (*Ratters norvegius*) weighing 180 to 200 g (100 days old) maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethical Committee(IAEC no: BRULAC/SDCH/SIMATS/IAECL07-2019/028 dated 13.07.2019) were used in the present study. Animals were housed in polypropylene cages under specific humidity(65% +/- 5%) and temperature(21^0C+/-2^0C) with constant 12 hr light and 12 hr dark schedule at Biomedical Research Unit and Lab Animal Center(BRULAC), Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences,Chennai-600077.they were fed with standard rat pellet diet (Lipton India, Mumbai, India).

Induction Of Type 2 Diabetes

Adult males rats were made diabetic (type-2) by a single intraperitoneal injection of streptozotocin (35 mg/kg body weight) after feeding the animals with high fat diet containing 3% of cholesterol,1% of cholic acid,30% of coconut oil,66% of standard rat feed and 30% of sucrose feeding through drinking water 25% for 60 days. After 60 days, all the animals were subjected to fasting blood glucose level. The rats which had the fasting sugar level above 120mg/dl, were considered to be type-2 diabetic (Jayaraman et al., 2021).

Experimental Design

Wistar strains of adult male albino rats (150-180 days) with 180-200 g body weight were split into five groups randomly. each group was consisting of 6 animals. Group I was served as control rats; Group II served as type-2 diabetic rats; Group III animals were served as piperine treated rats (40mg/kg b. wet); Group IV animals were served as metformin treated type-2 diabetic rats; Group V served as Piperine treated control rats. Piperine was treated orally, for 30 days, once in a day, orally. Piperine was dissolved in corn oil due its lipid soluble in nature. In order to check cytotoxicity, normal control rats were treated with same amount of corn oil. None of the animals showed any significance in the parameters studied.

After 30 days of treatment, all the animals were fasted overnight and on the next day, animals were checked. Then the animals were anaesthetised with 40 mg of sodium thiopentone per kg body weight. Through cardiac puncture, blood was collected and sera were separated and kept at -80°C.to clear the blood from various organs,20 ml of isotonic sodium chloride solution was perfused by way of the left ventricle. Adipose tissues were immediately dissected and utilised for the further study.

Fasting Blood Glucose (FBG)

Blood glucose was estimated using on-call blood glucose test strips (ACON) after overnight fasting. Blood was collected by pricking the tip of the rat tail and results are expressed as mg/dl (Babu et al., 2020).

Gene expression analysis

mRNA expression analysis of Nrf2,Keap1 and B actin genes by RT-PCR

Total RNA, 2µg was used for reverse transcriptase polymerase chain reaction(RT-PCR) analysis.RT-PCR was carried out using a two-step RT-PCR kit. In the first step,complementary DNA(cDNA) will be made from an mRNA template using Oligod T,dNTP's and reverse transcriptase. The components were transcriptase buffers for an hour at 37°C.After cDNA conversion,standard PCR was carried out using gene specific oligonucleotide primers by the initial PCR activation at 95°C for 5 mins. The three step PCR cycles consisted of denaturation at 95°C for 2 min,annealing at 60°C 30s and extension at 73°C for 30s.The PCR amplification was carried out for 30 cycles and to ensure that the products are extended completely,a final extension at 73°C for 5 min was

carried out. Gene- specific oligonucleotide primers for the house-keeping gene,B actin was added to the same PCR reaction vial and co-amplified. The following primers were used for gene expression analysis: Nrf2-FW: 5'-TCACACGAGATGAGCTTAGGGCAA-3', RW: 5'-TACAGTTCTGGG CGGCGACTT TAT-3'; β-actin-FW:5'- AGCTGCGTTT TACACCCTTT-3', RW-5'- AA GCCATGCCAATGTTGTCT-3'. The expression of β-actin mRNA was used as a reference gene to normalize data.

Statistical Investigation

Graphpad Prism 8.0 was used for statistical calculations. Mean values with SEM were presented. One-way ANOVA was used in statistical studies, and then the Newman-Keuls assessment for numerous comparisons. Statistics were deemed significant at $p< 0.05$.

3. Results and Discussion

Effect of piperine on the fasting blood glucose in high fat diet and sucrose-induced type-2 diabetic rats

Fasting blood glucose (FBG) is a crucial parameter in the diagnosis, management, and monitoring of type 2 diabetes mellitus. It serves as a fundamental tool for assessing glycemic control and has several important implications in the context of diabetes care. Regular monitoring of FBG levels helps healthcare providers and individuals evaluate the effectiveness of treatment regimens, including lifestyle modifications and medication therapy. In the present study, high fat diet and sucrose-induced rats showed a significant increase ($p<0.05$) in the levels of fasting blood glucose compared to control (Fig. 1). However, T2DM rats treated with 40mg dose of piperine orally improved the glycemic control whose effects were found to be equal to that of the metformin level.

Figure 1

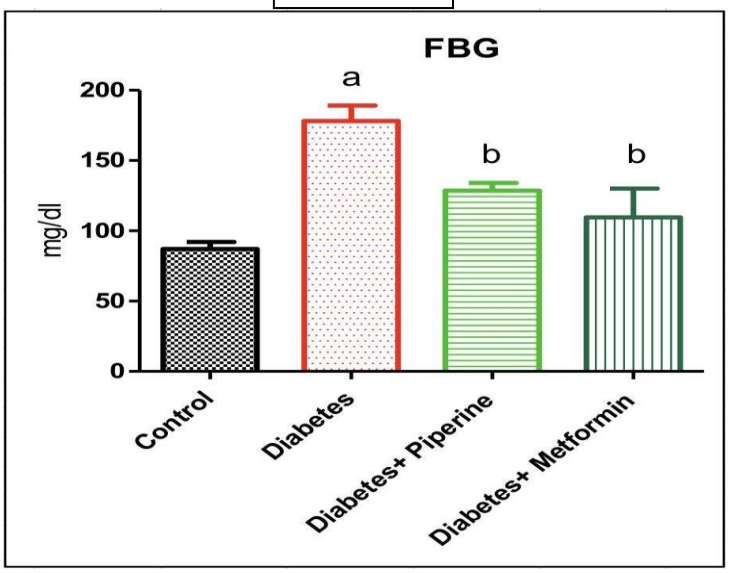

Figure 1: The effect of piperine on FBG in control and HFD induced T2DM rats. The numbers are as the mean ± SEM for six rats in each group.a-compared with control; b-compared with T2DM induced groups.

Effect of piperine on Nuclear factor erythroid 2-related factor 2 (Nrf-2) in the adipose tissue of high fat diet and sucrose-induced type-2 diabetic rats

Nrf2 (Nuclear factor erythroid 2-related factor 2) is a transcription factor that plays a central role in cellular defense against oxidative stress and inflammation. It is involved in the regulation of antioxidant and detoxification enzymes, making it a crucial component of the body's defense against oxidative damage. In the context of diabetes, Nrf2 has gained significant attention for its potential role in diabetes management and prevention. In this study, high fat treatment showed detrimental effects of the mRNA expression of Nrf-2 compared to healthy control rats but metformin and piperine treatment with oral dose of 40 mg, improved the expression of Nrf2-gene significantly ($p<0.05$) suggesting that piperine has significant role on antioxidant signalling (Figure 2).

Figure 2

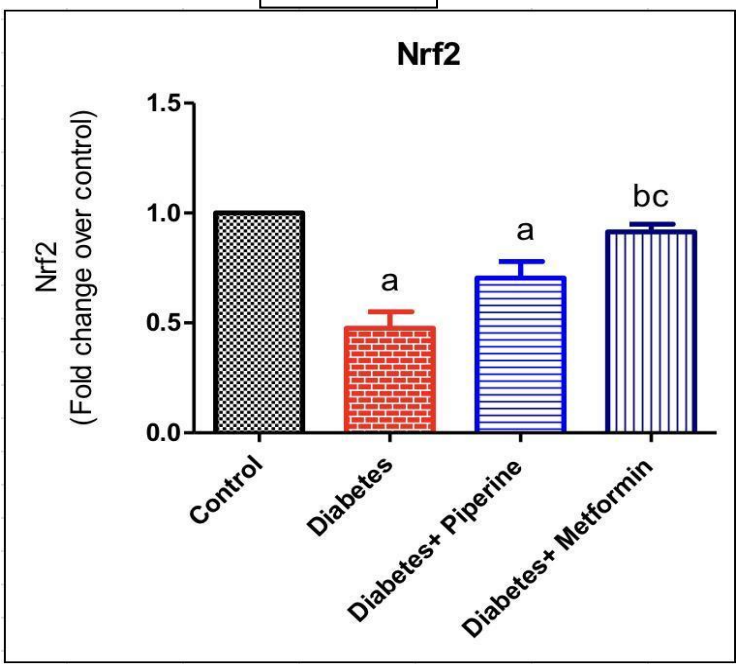

Figure 2: Effect of piperome on mRNA expression of Nrf-2 in adipose tissue of type-2 diabetic rats. The mRNA expression of Nrf-2 was assessed by Real Time-PCR. mRNA expression is given in fold change. Each bar represents mean ± SEM (n = 6). Significance at $p < 0.05$. a Significantly different from the control group; b significantly different from the diabetic group and c significantly different from the piperine treated diabetic group.

Effect of piperine on Keap1 mRNA expression in the adipose tissue of high fat diet and sucrose-induced type-2 diabetic rats

Keap1 (Kelch-like ECH-associated protein 1) is a protein that plays a significant role in regulating the activity of the transcription factor Nrf2 (Nuclear factor erythroid 2-related factor 2). The Keap1-Nrf2 system is a critical pathway involved in the cellular defense against oxidative stress and inflammation. While much of the research on Keap1 has focused on its role in various diseases, including cancer, cardiovascular disease, and neurodegenerative disorders, its involvement in diabetes has also been investigated. In this context, the Keap-1 mRNA expression was measured using Real-Time PCR method and amplified using gene specific primers. As depicted in the figure 3, there was a significant increase in the Keap-1 mRNA levels were found while piperine treatment did not have any significant effects on the Keap-1 molecules expression. However, metformin treatment showed significant effects on Keap -1 expression ($p<0.05$).

Figure

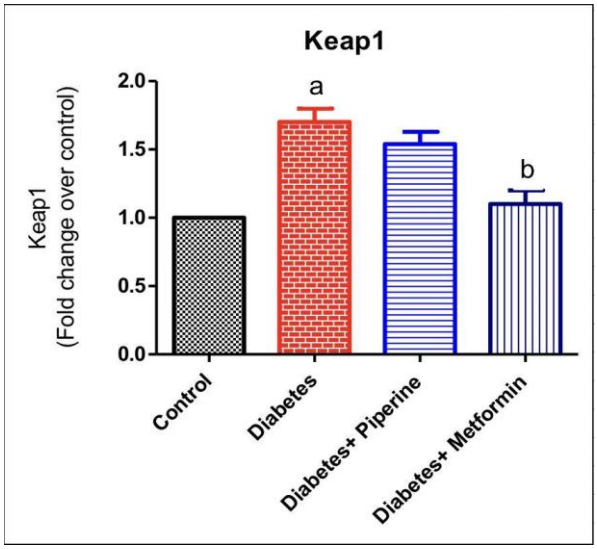

Figure 3: Effect of piperome on mRNA expression of Keap-1 in adipose tissue of type-2 diabetic rats. The mRNA expression of Nrf-2 was assessed by Real Time-PCR. mRNA expression is given in fold change. Each bar represents mean ± SEM (n = 6). Significance at $p < 0.05$. a Significantly different from the control group; b significantly different from the diabetic group and c significantly different from the piperine treated diabetic group

The occurrence of T2DM stems from the inability of the body's cells to effectively metabolize sugar, which can be attributed to either inadequate insulin construction by the pancreas or ineffective utilization of body insulin. The condition is characterized by chronic hyperglycemia and changes in the metabolism of macromolecules resulting from flaws in insulin production and activity (Salehi et al., 2019). T2DM constitutes the majority of diabetes cases, making up approximately 90%. Preventing and managing T2DM involves adopting a comprehensive approach that emphasizes the promotion of a healthy lifestyle and regular exercise. For centuries, medicinal plants have been utilized to address a wide range of health conditions, and one such plant is Piper nigrum. This plant contains commonly used spice called piperine, a phytochemical that imparts the spice with its characteristic bitter taste. piperine derived from the pepper plant, has been extensively studied for its potential benefits (Smilkov et al., 2019; Haq et al., 2021). However, it is unknown whether piperine can modulate HFD-mediated impairment in Nrf-2/Keap-1 mediated signaling, one of the major causes of T2DM. Hence, we focused on the study investigating the possible role of piperome in reducing diabetes risks by exploring its mechanism in adipose tissue.

In the present study piperine treatment, efficiently reversed fasting blood sugar levels in diabetic rats to those of the control group. However, diabetic rats treated with piperine showed reduced the glucose level which was near to that of the control groups. These findings highlight the potential of piperine as a promising therapeutic agent T2DM. In this regard, it has been on invitro model that piperine has potential to modulate glucose metabolism. It may improve glucose uptake in muscle cells and enhance insulin sensitivity, which could help regulate blood sugar levels (Srinivasan, 2007).

In order to check if this effect is mediate through Nrf-2/keap-1 antioxidant signaling mechanism, we performed gene expression analysis using gene specific primers by q-RT-PCR analysis. Nrf2 regulates the expression of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which can help mitigate oxidative stress in adipocytes. Reducing oxidative stress in adipose tissue is important for improving insulin sensitivity. In this study, high fat treatment showed detrimental effects of the mRNA expression of Nrf-2 compared to healthy control rats but metformin and piperine treatment with oral dose of 40 mg, improved the expression of Nrf2-gene significantly this tudy clearly show that piperine has significant role on antioxidant signalling.

Keap1 is known for its role in regulating Nrf2 activity. Under normal conditions, Keap1 binds to Nrf2 and promotes its degradation. Dysregulation of the Keap1-Nrf2 pathway can result in reduced antioxidant defense and increased susceptibility to oxidative damage in individuals with diabetes (Shin et al., 2009; Tanaka et al.,

2012). In order to achieve this, we isolated RNA from adipose tissue and amplified the Keap-1 gene expression in fold change. Though the standard drug metformin showed a significant effect on Keap-1, piperine did not exhibit any significant change and this study suggest that piperine does not pay a role on Keap-1 expression. Taken together, our study clearly implies that piperine has the potency to reduce hyperglyemia.

4. Conclusion

Diabetes is characterized by chronic hyperglycemia and is associated with various metabolic abnormalities, including oxidative stress. The Nrf2-Keap1 pathway plays a critical role in antioxidant signaling and has garnered attention as a potential target for managing oxidative stress in diabetes. Additionally, various natural compounds have been studied for their ability to activate the Nrf2-Keap1 pathway and mitigate oxidative stress in diabetes. Results of the present findings clearly showed that piperine potentially regulate antioxidant signaling pathway (Nrf-2/keap-1) in adipose tissue thereby it reduces high fat diet and sucrose-induced oxidative stress mediated the development type-2 diabetes to its antioxidant potential. Hence, the present study concludes that piperine can be considered as an effective natural compound for the treatment of type-2 diabetes. To the beset of our knowledge, the present findings are the first of its kind providing an experimental invivo evidence against HFD-sucrose-induced T2DM model. Further studies on the protein expression and downstream signaling molecules of Nrf-2/Keap-1 and human cell line, need to be studied prior to clinical trials.

Conflict of Interest :

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

Acknowledgement :

The authors express their gratitude to Saveetha Dental College & Hospitals for supporting and for successful completion of this project.

Source of funding:

The present project is funded by

1. Saveetha Institute of Medical and Technical Sciences
2. Saveetha Dental College and Hospitals
3. Saveetha University
4. Sri Amutha Dental Care,Thuraiyur

Author Contribution :

A) Mounithaa N - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B) Dr Selvaraj J - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

C) Dr.V.Vishnupriya - contributed in study design, guiding the research work, manuscript correction.

D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.

E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

References

Ampawong S, Isarangkul D, Aramwit P, & Khunkitti W. (2016). Effects of piperine on glucose uptake in muscle cells: A mechanism study. *Integrative Medicine, 15*(2), 34-41.

Babu S, Krishnan M, Rajagopal P, et al. (2020). Beta-sitosterol attenuates insulin resistance in adipose tissue via IRS-1/Akt mediated insulin signaling in high-fat diet and sucrose-induced type-2 diabetic rats. *European Journal of Pharmacology, 873*, 173004.

Dong B, Young M, Liu X, Singh AB, & Liu J. (2017). Regulation of lipid metabolism by obeticholic acid in hyperlipidemic hamsters. *Journal of Lipid Research, 58*(2), 350-363.

Giacco F, & Brownlee M. (2010). Oxidative stress and diabetic complications. *Circulation Research, 107*(9), 1058-1070.

Haq IU, Imran M, Nadeem M, Tufail T, Gondal TA, & Mubarak MS. (2021). Piperine: A review of its biological effects.

Phytotherapy Research, *35*(2), 680-700.

Jayaraman S, Devarajan N, Rajagopal P, Babu S, Ganesan SK, Veeraraghavan VP, Palanisamy CP, Cui B, Periyasamy V, & Chandrasekar K. (2021). β-Sitosterol Circumvents Obesity Induced Inflammation and Insulin Resistance by down-Regulating IKKβ/NF-κB and JNK Signaling Pathway in Adipocytes of Type 2 Diabetic Rats. *Molecules*, *26*(7), 2101.

Maity TK, Mandal SC, Saha BP, & Pal M. (2005). Piperine, the active principle of Piper nigrum, is a potential antidiabetic agent. *Journal of Natural Products*, *68*(2), 285-286.

Prasad M, Jayaraman S, Eladl MA, El-Sherbiny M, Abdelrahman MAE, Veeraraghavan VP, Vengadassalapathy S, Umapathy VR, Jaffer Hussain SF, Krishnamoorthy K, Sekar D, Palanisamy CP, Mohan SK, & Rajagopal P. (2022). A Comprehensive Review on Therapeutic Perspectives of Phytosterols in Insulin Resistance: A Mechanistic Approach. *Molecules*, *27*(5), 1595.

Salehi B, Ata A, V Anil Kumar N, et al. (2019). Antidiabetic Potential of Medicinal Plants and Their Active Components. *Biomolecules*, *9*(10), 551.

Sanjay Varshan M, Lavanya Prathap, Selvaraj Jayaraman, & Preetha. S. (2023). Anti-proliferative effect of endogenous dopamine replica in human lung cancer cells (A549) via PI3K and Akt signaling molecules. *Journal of Pharmaceutical Negative Results*, 1380-1386.

Shin S, Wakabayashi J, Yates MS, et al. (2009). Role of Nrf2 in prevention of high-fat diet-induced obesity by synthetic triterpenoid CDDO-imidazolide. *European Journal of Pharmacology*, *620*(1-3), 138-144.

Smilkov K, Ackova DG, Cvetkovski A, Ruskovska T, Vidovic B, & Atalay M. (2019). Piperine: Old Spice and New Nutraceutical? *Current Pharmaceutical Design*, *25*(15), 1729-1739.

Srinivasan K. (2007). Black pepper and its pungent principle-piperine: a review of diverse physiological effects. *Critical Reviews in Food Science and Nutrition*, *47*(8), 735-748.

Tabish SA. (2007). Is Diabetes Becoming the Biggest Epidemic of the Twenty-first Century? *International Journal of Health Sciences (Qassim)*, *1*(2), V-VIII.

Vishaka, S, Sridevi G, & Selvaraj, J. (2022). An in vitro analysis of the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. *Journal of Advanced Pharmaceutical Technology & Research*, *13*(Suppl 2), S505–S509.