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Effect of Natural Flavonoid Apigenin in Lowering High Glucose-Induced Insulin Resistance via Targeting PI3K/AKT Pathway in 3T3-L1 Adipocytes – Evidence Through an *In-vitro* and *In-silico* Approach

Pranav Raaj, R.[1], Selvaraj Jayaraman[2*], Vishnu Priya Veeraraghavan[2]
[1]*Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai--602105, India*
[2]*Centre of Molecular Medicine and Diagnostics (COMManD), Department of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai 600077, India*

Abstract

Diabetes mellitus, characterized by elevated blood glucose levels resulting from insulin deficiency or resistance, poses a significant global health challenge. With its increasing prevalence and substantial impact on morbidity, mortality, and healthcare costs, effective strategies for managing diabetes are urgently needed. Natural flavonoid such as apigenin, has emerged as potential therapeutic agent due to their antioxidant, anti-inflammatory anti-diabetic properties but mechanism of action is not known. The study was aimed at assessing the role of apigenin on PI3K/AKT/GLUT4 pathway in 3T3-L1 adipocytes. Invitro alpha amylase and alpha glucosidase inhibitory activity was measured by spectrophotometric methods. Cytotoxicity was assessed by MTT assay. Further, gene expression analysis was done by Real Time-PCR. In order to confirm the exact binding interaction of apigenin with PI3K/Akt/GLUT4 signaling, molecular docking analysis was also performed. Results of this study showed that apigenin significantly reduced alpha amylase and alpha glucosidase inhibitory activity in a dose-dependent fashion. q-PCR analysis showed that apigenin significantly improved mRNA expression of insulin signaling molecules (IR, IRS-1, PI3K, Akt and GLUT4) in high glucose-induced 3T3-L1 adipocytes cell line. Molecular docking analysis evidenced that apigenin confirmed possible role of apigenin that regulates insulin metabolic signaling in adipocytes. Overall, apigenin holds promise as a natural flavonoid with potential therapeutic value in combating diabetes and its complications, underscoring the importance of continued research to unlock its full therapeutic potential and pave the way for effective diabetes management strategies.

Keywords: *Adipocytes, Anti-diabetic, Apigenin, Diabetes Mellitus, Hyperglycaemic, Insulin Signalling, Public Health.*

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by high blood glucose levels resulting from deficiencies in insulin action or production, or both. It poses significant challenges to global public health due to its impact on morbidity, mortality, and healthcare expenditures. The prevalence of diabetes has been increasing worldwide, with the International Diabetes Federation estimating that 463 million individuals aged 20 to 79 had diabetes in 2019, a number projected to reach 700 million by 2045. The majority of diabetes cases are attributed to type 2 diabetes mellitus (T2DM), though type 1 diabetes mellitus (T1DM) is also on the rise, particularly among children and teenagers [1, 2]. The rise in diabetes incidence is influenced by various

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****Corresponding Author: selvarajj.sdc@saveetha.com***

factors including aging populations, unhealthy dietary habits, sedentary lifestyles, urbanization, and obesity. Diabetes not only adversely affects individual health but also imposes a substantial financial burden on healthcare systems and society as a whole. Complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy further exacerbate the morbidity and mortality associated with diabetes. Efforts to combat the diabetes pandemic involve preventive interventions targeting modifiable risk factors, promotion of healthy lifestyles, early detection and diagnosis, and improved access to quality healthcare services. Public health interventions aimed at managing and preventing diabetes are crucial for mitigating the impact of this chronic illness on individuals and global healthcare systems [3,4]. Natural flavonoids, including apigenin, quercetin, and kaempferol, have garnered attention for their potential role in controlling hyperglycemia associated with diabetes mellitus. These compounds, abundant in fruits, vegetables, drinks, and herbs, exhibit antioxidant, anti-inflammatory, and anti-diabetic properties. Flavonoids influence hyperglycemia by altering insulin signaling pathways, enhancing insulin sensitivity, promoting glucose absorption, and protecting pancreatic beta cells from oxidative stress-induced damage [5,6]. Specifically, apigenin has shown promise in modulating the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, which plays a crucial role in insulin-mediated glucose metabolism. *In vitro* studies have demonstrated apigenin's ability to enhance insulin sensitivity and glucose uptake in insulin-resistant cells, potentially through activation of the PI3K/AKT pathway. Additionally, apigenin's antioxidant properties may help mitigate oxidative stress and preserve beta cell function and insulin secretion.

Various research methodologies, including *in vitro* experiments, animal studies, epidemiological analyses, and clinical trials, are employed to investigate the therapeutic potential of flavonoids in managing hyperglycemia and diabetes. *In vitro* studies involve treating cells with flavonoid compounds and evaluating their effects on insulin sensitivity, glucose uptake, and related signaling pathways. Animal studies assess the impact of flavonoid supplementation on parameters such as blood glucose levels, insulin sensitivity, and pancreatic beta cell activity in diabetic animal models. Epidemiological studies examine the relationship between dietary flavonoid consumption and the risk of diabetes or its complications in human populations. Clinical trials evaluate the safety and efficacy of flavonoid supplementation as an adjunctive therapy for diabetes management, assessing outcomes such as insulin sensitivity, lipid profiles, oxidative stress markers, and glycemic control. *In silico* approaches, such as molecular docking, provide valuable insights into the interactions between apigenin and components of the PI3K/AKT pathway, aiding in the prediction of potential therapeutic effects. These computational techniques expedite the drug development process by elucidating molecular mechanisms, predicting drug-target interactions, and optimizing drug candidates prior to experimental validation. Overall, natural flavonoids, including apigenin, hold promise as adjunctive therapies for diabetes management by modulating insulin signaling pathways and glucose homeostasis. However, further research is needed to fully understand their safety, efficacy, and optimal dosage regimens, including well-designed clinical studies. By employing multidisciplinary research approaches, scientists aim to develop evidence-based strategies for harnessing the therapeutic potential of flavonoids in combating diabetes and associated metabolic disorders [7,8].

Materials and Methods

Chemical and Reagents

All chemicals and reagents used in this research were of molecular and analytical grade, sourced from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India; Sigma Chemical Company (St. Louis, MO, USA); and MP Biomedicals (Santa Ana, CA, USA). IR, IRS1, PI3K, AKT, GLUT4, and β-actin gene-specific primers were obtained from Eurofins Scientific, Bengaluru, India. The phytocompound Apigenin was purchased from Sigma Chemical Company (St. Louis, MO, USA).

Assessment of Antidiabetic Activity

Alpha amylase inhibitory activity

50µl of phosphate buffer, 10µl of alpha amylase and 20µl of apigenin at different concentrations (0.1-0.5 mg/ml) is preincubated at 20^0C for 20mins. Then 20µl of 1% starch was added and incubated at 37^0C for 30mins. 100µl of DNS colour reagent was added and boiled for 10mins. Optical density will be measured at 540nm. In this study acarbose was used as standard.

$$Inhibition\ \% = \frac{Absorbance\ of\ Control - Absorbance\ of\ Test\ Sample}{Absorbance\ of\ Control} \times 100$$

Alpha glucosidase inhibitory activity

50µl of phosphate buffer, 10µl of alpha glucosidase and 20µl of apigenin extract at different concentrations (0.1-0.5 mg/ml) is preincubated at 37^0C for 15mins. Then 20µl of P-NPG will be added and incubated at 37^0C for 20mins. The reaction will be stopped by adding 50µl of sodium bicarbonate. The absorbance will be measured at 405nm. In this study acarbose was used as standard.

$$Inhibition\ \% = \frac{A\,bsorbance\ of\ Control - Absorbance\ of\ Test\ Sample}{Absorbance\ of\ Control} \times 100$$

Assessment of *In Vitro* Anti-oxidant Activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

Scavenging of DPPH radical was assessed by the method of [9]. Briefly, DPPH solution (1.0 ml) was added to 1mg/ml of Apigenin at different concentrations (100, 200, 300, 400, and 500 µg/ml). The mixture was kept at room temperature for 50 min and the activity was measured at 517 nm. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as standard. The capability to scavenge the DPPH radical was calculated using the following formula: DPPH radicals scavenged (%) = (Control OD − Sample OD/Control OD) × 100.

$$Inhibition\ \% = \frac{Absorbance\ of\ Control - Absorbance\ of\ Test\ Sample}{Absorbance\ of\ Control} \times 100$$

In Vitro Cell Line Study

Procurement and culture of Human Adipocyte cell line (3T3 L-1) The 3T3-L1 cell line was acquired from The National Centre for Cell Science (NCCS), located in Pune, India, and cultivated following the prescribed cell culture protocols. In summary, the 3t3 L1 cells were cultured in Minimum Essential Medium (MEM**)** supplemented with 10% fetal bovine serum (FBS) under standard conditions of 37°C temperature and 5% CO_2 atmosphere. Culturing was carried out at 37°C in a humidified atmosphere containing 5% CO_2 to provide optimal growth conditions for the 3T3 L1cells. This optimal culture environment facilitated the robust growth and maintenance of the 3T3 L1 adipocyte cell line, ensuring its viability and suitability for subsequent experimental procedures.

% Growth inhibition = (1 - OD extract treated)/OD negative control x 100.

Gene Expression Analysis by Real Time-PCR

Gene expression levels were examined using real-time PCR. The total RNA was isolated by using TriR Reagent (Sigma). Total RNA (2 µg) from each sample was reverse transcribed using a commercial Superscript III first strand cDNA synthesis kit (Invitrogen, USA) according to the manufacturer's protocol. Real time-PCR was carried out in a MX3000p PCR system

(Stratagene, Europe). Reaction was performed using MESA Green PCR master mix (It contains all the PCR components along with SYBR green dye) Eurogentec, USA. The specificity of the amplification product was determined by melting curve analysis for each primer pairs. The data were analyzed by comparative CT method and the fold change is calculated by 2−CT method using CFX Manager Version 2.1 (Bio Rad, USA). Details of gene specific primers used is given in the table1.

Table 1. List of Primers used in this Study

Gene	Forward Primer	Reverse Primer
IR	5'-GTGAAGATGGAAGGAAAGA-3'	5'-CAGAGTGAAGGAATGACAGG-3'
IRS1	5'-TGGCAGTGAGGATGTGAAAC-3'	5'-CTTGGATGCTCCCCCTAGAT-3'
PI3K	5'- CTCTCCTGTGCTGGCTACTGT -3'	5'- GCTCTCGGTTGATTCCAAACT -3'
AKT	5'- ATCCCCTCAACAACTTCTCAGT -3'	5'- CTTCCGTCCACTCTTCTCTTTC -3'
GLUT4	5'-GAGCCTGAATGCTAATGGAG-3'	5'-GAGAGAGAGCGTCCAATGTC-3'
β-actin	5'-CCTGAGGCTCTTTTCCAGCC-3'	5′-TAGAGGTCTTTACGGATGTCAACGT-3′

Molecular Docking Analysis

The binding interactions of a compound with specific target proteins were investigated using the molecular docking software PyRx. Crystal structures of these target proteins were retrieved from the Protein Data Bank (PDB). Throughout the docking process, a grid box measuring 90 Å × 90 Å × 90 Å, with a grid spacing of 0.45 Å, was employed to facilitate accurate ligand-protein interaction prediction. Docking calculations were carried out utilizing the Lamarckian genetic algorithm (LGA), with 100 genetic algorithm runs executed to ensure comprehensive exploration of the conformational space. Post-docking analysis was conducted to identify and characterize high-pose interactions between the compound of interest (Apigenin) and the apoptosis-regulating target proteins. Additionally, the binding affinities of the ligand towards the receptors were meticulously evaluated to elucidate the binding mode. The resulting 3D structures of the ligand-protein complexes from the docking simulations were visualized and analyzed using BIOVIA Discovery Studio, enabling a detailed examination of the molecular interactions and the binding conformations. This comprehensive approach provides insights into the potential mechanisms of action and therapeutic relevance of the compound in modulating apoptotic pathways through its interactions with specific protein targets.

ADME Properties of Apigenin

Our research incorporated in silico pharmacokinetic analysis to explore the Absorption, Distribution, Metabolism, and Excretion (ADME) properties of the apigenin compound. We predicted key parameters including molecular weight, topological polar

surface area (TPSA), miLogP, the number of rotatable bonds, and the counts of hydrogen donor and acceptor atoms, all in accordance with Lipinski's criteria. This assessment was conducted using a web-based tool available at www.swissadme.ch, which is specifically designed to compute physicochemical descriptors and forecast pharmacokinetic properties for small molecules.

Statistical Analysis

The data presented are represented as the means ± standard deviation (SD) derived from three independent experiments conducted in triplicate. Statistical analysis was carried out using one-way ANOVA, with a significance level set at $p<0.05$ to indicate statistically significant results.

Results

Alpha Amylase Inhibitory Activity of Apigenin

An essential technique in drug discovery and development, especially when looking for new treatments for obesity and diabetes, is the alpha-amylase inhibition assay. Based on the provided data in Table 1, it appears to represent the results of an alpha-amylase inhibition assay conducted with different concentrations of an extract or compound. The concentrations tested range from 12.5 µg/ml to 200 µg/ml. For each concentration, the mean percentage inhibition of alpha-amylase activity is reported, along with the standard deviation where applicable. Additionally, the mean percentage inhibition of a positive control or reference compound is provided for comparison. The results indicate a dose-dependent increase in the inhibition of alpha-amylase activity with increasing concentrations of the extract or compound. At the lowest concentration tested (12.5 µg/ml), the mean percentage inhibition is 31.13%, while at the highest concentration (200 µg/ml), it reaches 80.15%. This trend suggests that the extract or compound exhibits significant inhibitory activity against alpha-amylase.

Comparing the mean percentage inhibition of the extract or compound with that of the positive control reveals its effectiveness relative to a known inhibitor. The extract or compound demonstrates comparable or even superior inhibition of alpha-amylase activity compared to the positive control across all concentrations tested. These findings suggest that the extract or compound may possess promising potential as an alpha-amylase inhibitor, warranting further investigation into its efficacy, mechanism of action, and potential therapeutic applications, particularly in the management of hyperglycemia and related metabolic disorders as opposed to alpha-amylase. The efficiency of the extract or compound in comparison to a known inhibitor can be determined by comparing its mean percentage inhibition with that of the positive control. In comparison to the positive control, the extract or compound exhibits equivalent or even greater inhibition of alpha-amylase activity at all tested doses. These results (Table 2 & Figure 1) imply that the extract or compound may have promising potential as an alpha-amylase inhibitor, indicating the need for additional research to determine its effectiveness, mode of action, and possible therapeutic uses, especially in the treatment of hyperglycemia and associated metabolic disorders.

Table2. Shows Mean and Standard Deviation of Alpha Amylase Inhibitory Activity of Apigenin

Conc (µg/ml	Mean (Std % Inhibition)	Mean (Extract % Inhibition)
12.5	31.13	23.29
25	40.08	33.51

50	53.84	41.07
100	69.89	49.75
200	80.15	59.83

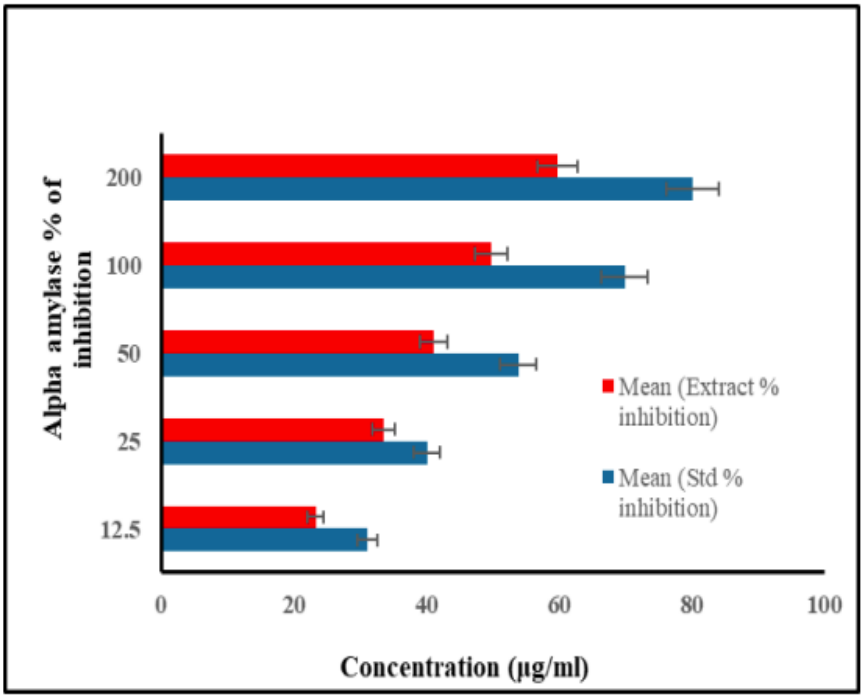


Figure 1. α-amylase Inhibitory Activity of Apigenin

Alpha Glucosidase Inhibitory Activity of Apigenin

An analytical method used in laboratories to assess a compound's capacity to impede the activity of the alpha-glucosidase enzyme is the alpha-glucosidase inhibition assay. In the small intestine, this enzyme is in charge of dissolving complex carbs into simpler sugars like glucose. Compounds can lower postprandial blood glucose levels by slowing down the digestion and absorption of carbs by blocking alpha-glucosidase. Alpha-glucosidase enzyme is usually purchased commercially or isolated from natural sources for use in the experiment. After that, the enzyme is combined with a substrate—typically a man-made chromogenic or fluorescent substrate—that, when cleaved by the enzyme, yields a detectable signal. After the test chemical or extract is added to the reaction mixture, the amount of inhibition is assessed by comparing the enzyme activity in the compound's presence compared to its absence. Numerous techniques, such as spectrophotometry, fluorometry, or high-performance liquid chromatography (HPLC), can be used to measure the activity of an enzyme. Based on the variation in enzyme activity between the test and control samples, the degree of inhibition is given as a percentage. In the process of finding and developing new drugs, the alpha-glucosidase inhibition assay is frequently employed, especially when looking for novel anti-diabetic medications. Potent inhibitors of alpha-glucosidase activity that show promise *in vitro* could be tested for safety and effectiveness in animal models before moving on to clinical trials as possible diabetes mellitus therapies. Furthermore, this assay can be used to screen synthetic chemicals, natural products, and pharmacological libraries in order to find new inhibitors of alpha-glucosidase activity (Table 3 & Figure 2).

Table 3. Showing Mean and Standard Deviation of Alpha Amylase Inhibitory Activity of Apigenin

Conc (µg/ml	Mean (Std % Inhibition)	Mean (Extract % Inhibition)
15	29.32	20.33
30	40.08	31.51
60	58.84	44.07
120	72.89	53.75
240	85.15	69.98

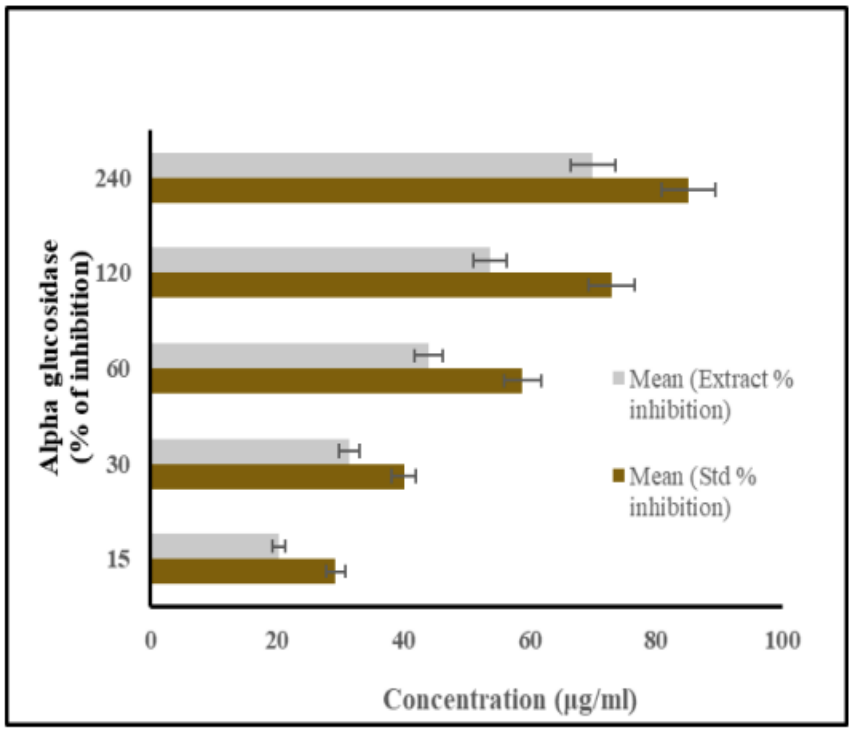


Figure 2. α-glucosidase Inhibitory Activity of Apigenin

The (Table 3 & Figure 2) shows the outcomes of an alpha-glucosidase inhibition experiment carried out using various test drug or extract quantities. The measured concentrations vary from 15 µg/ml to 240 µg/ml. The percentage inhibition of alpha-glucosidase activity for each concentration is given, along with a corresponding positive control result for context. As with the alpha-amylase inhibition experiment, the data show that the inhibition of alpha-glucosidase activity increases with increasing concentrations of the test chemical or extract in a dose-dependent manner. The percentage inhibition is 29.32% at the lowest dose (15 µg/ml) and 85.15% at the maximum concentration (240 µg/ml). This pattern implies that the substance or extract has a strong alpha-glucosidase inhibitory effect of the positive control shows how effective it is in comparison to a recognized inhibitor. In comparison to the positive control, the chemical or extract exhibits equivalent or even greater inhibition of alpha-glucosidase activity at all tested doses. These results imply that the extract or chemical may have intriguing potential as an alpha-glucosidase inhibitor, necessitating additional research into its effectiveness, mode of action, and its therapeutic uses in the treatment of diabetes and hyperglycemia.

DPPH Radical Scavenging Activity of Apigenin

The assay known as DPPH (2,2-diphenyl-1-picrylhydrazyl) is a popular technique utilized to evaluate the antioxidant capacity of substances or mixtures. When DPPH, a stable free radical, combines with an antioxidant, it gets reduced and turns yellow instead of purple. A DPPH solution is combined with the test substance or extract in the assay, and the absorbance at a particular wavelength—typically 517 nm decreases over time. The DPPH assay is also useful for determining a compound's or extract's antioxidant potential and evaluating how effective it is in comparison to established antioxidants or standards. Through assessing a compound's capacity to neutralize free radicals, scientists can learn more about its mode of action and structure-activity connections. Furthermore, antioxidant-rich formulations and goods can be developed and optimized with the use of the DPPH assay, which can improve the products' ability to prevent oxidative damage and advance general health and well-being (Table 4 & Figure 3).

Table:4. Showing Mean and Standard Deviation of DPPH Radical Scavenging Activity of Apigenin

Conc (µg/ml	Mean (Std % inhibition)	Mean (Extract % inhibition)
100	42.18	29.43
200	53.76	38.51
300	64.85	49.15
400	78.81	62.26
500	87.74	75.08

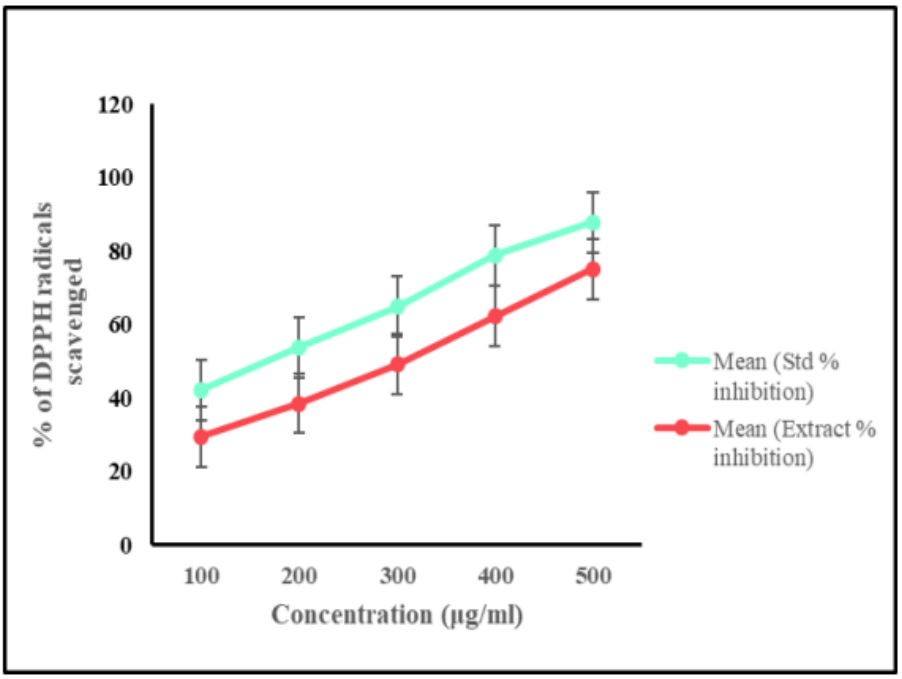


Figure 3. Antioxidant Activity of Apigenin

Table 4 & Figure3 show the outcomes of a DPPH radical scavenging test carried out with various test chemical or extract concentrations. The measured concentrations vary from 100 µg/ml to 500 µg/ml. The mean percentage inhibition of the DPPH radical is given at each concentration, together with the standard deviation if relevant. Furthermore, for comparison, the mean percentage inhibition of a reference or positive control chemical is

given. The increasing percentage suppression of the DPPH radical with increased concentrations of the chemical or extract suggests a dose-dependent increase in the antioxidant activity of the sample. The mean percentage inhibition is 42.18% at the lowest tested concentration (100 µg/ml) and 87.74% at the highest concentration (500 µg/ml). This pattern implies that the mixture extract has strong antioxidant properties and can efficiently scavenge DPPH radicals in a concentration-dependent way. The efficacy of the chemical or extract in comparison to a recognized antioxidant can be determined by comparing its mean percentage inhibition with that of the positive control. In comparison to the positive control, the chemical or extract exhibits equivalent or even greater antioxidant activity at all tested doses. These results imply that the molecule or extract may have promising potential as an antioxidant agent, indicating the need for additional research into its effectiveness, mode of action, and possible therapeutic uses in the treatment of disorders associated with oxidative stress (Table 4 & Figure 3).

Effects of Apigenin on Cytotoxicity in 3T3-L1 Adipocytes

As depicted in figure 4, apigenin treatment to normal adipocytes did not show cytotoxicity till 30µM concentration while at the concentrations of 40 and 50 µM concentration showed 10-20 % cytotoxicity. Hence, 30 µM was taken into consideration as an effective dose for further analysis.

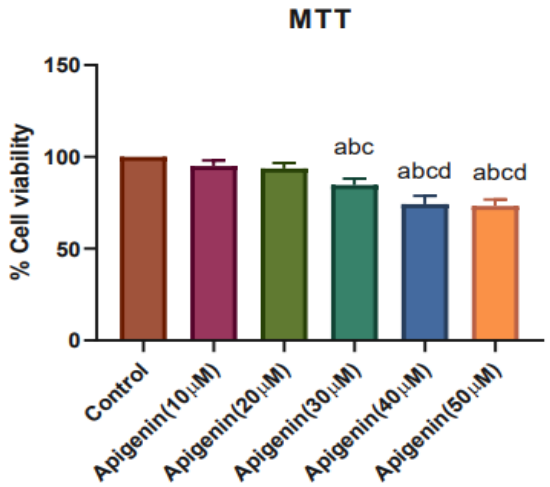


Figure 4. Effects of Apigenin on the Cytotoxicity in 3T3 L1 Adipocytes

Effects of Apigenin on the mRNA Expression of Insulin Signaling Molecules in 3T3-L1 Adipocytes

The depicted figures illustrate the expression levels of the IR, Akt, IRS-1, PI3k GLUT-4, genes across different concentrations of apigenin. Upon exposure to apigenin, there is a notable decrease in the expression levels of genes such as IR and GLUT-4, which are typically upregulated in diabetic states. This reduction suggests that apigenin may have a regulatory effect on these genes, potentially reversing the aberrant expression associated with diabetes. Conversely, the expression of genes like IRS-1, PI3K, Akt shows an increase with increasing concentrations of quercetin. While elevated expression of these genes is often linked to inflammation and insulin resistance in diabetes, the observed upregulation suggests a potential mechanism by which quercetin exerts its therapeutic effects (Figure 5A-5E). These findings underscore the promising therapeutic implications of quercetin in mitigating diabetic activity by modulating

the expression of critical genes involved in the disease process. Further investigation into the molecular mechanisms underlying these effects is warranted to fully elucidate the anti-diabetic properties of quercetin and its potential as a therapeutic agent for diabetes management.

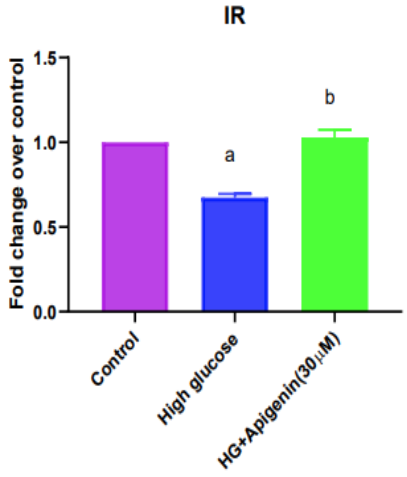


Figure 5A. Effects of Apigenin on IR mRNA in High Glucose Induced-adipocyte Cells

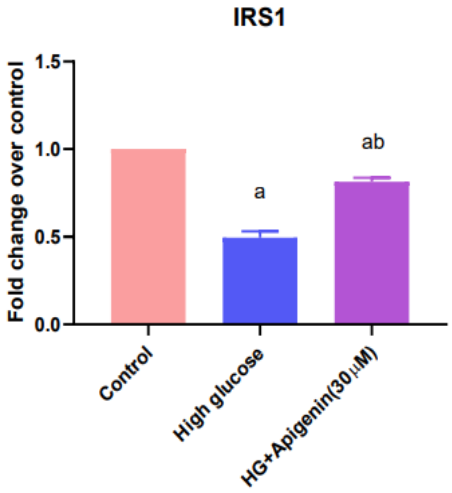


Figure 5B. Effects of Apigenin on IRS-1 mRNA in High Glucose Induced-adipocyte Cells

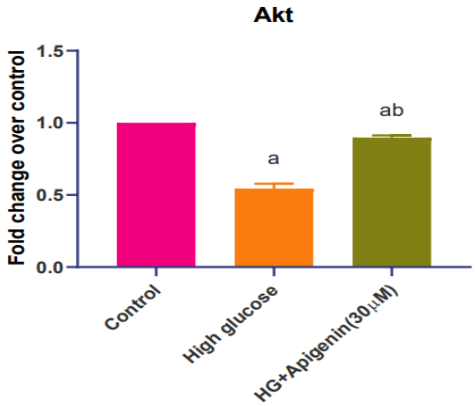


Figure 5C. Effects of Apigenin on Akt mRNA in High Glucose Induced-adipocyte Cells

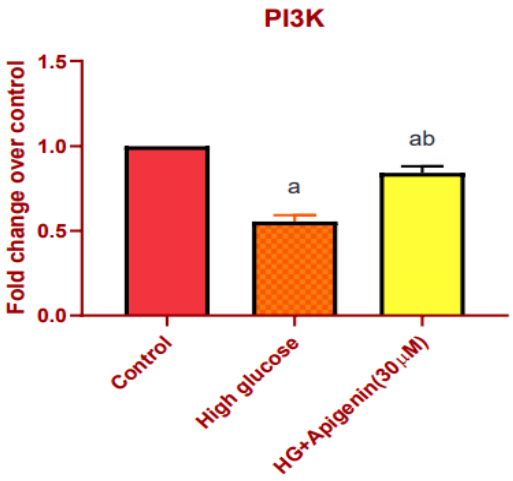


Figure 5D. Effects of Apigenin on PI3K mRNA in High Glucose Induced-adipocyte Cells

Gene expression for IR, Akt, IRS-1, and PI3K, Glut-4 by apigenin against 3T3-L1 adipocyte cell line. However, the figures demonstrate a notable decrease in the activity of both IR, IRS-1 and GLUT-4genes with increasing activity of Akt, PI3K with increasing concentrations of apigenin in relation with high glucose groups with comparison to the control group. These findings highlight the promising therapeutic implications of the apigenin mitigating diabetic activity by modulating the expression of critical genes associated with diabetic regulation. Further investigation into the molecular mechanisms underlying these effects is warranted to fully elucidate the antidiabetic property of apigenin (Figure 5A-5E).

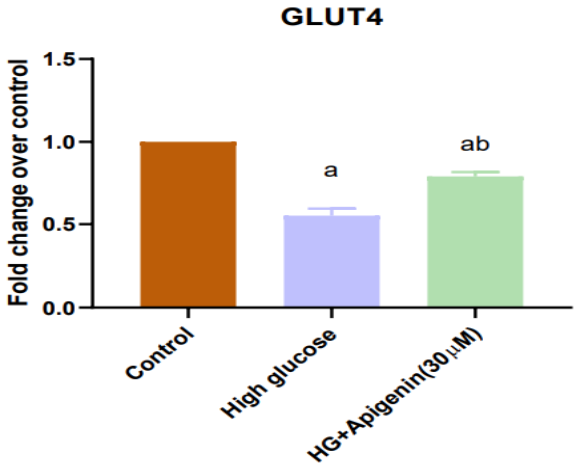
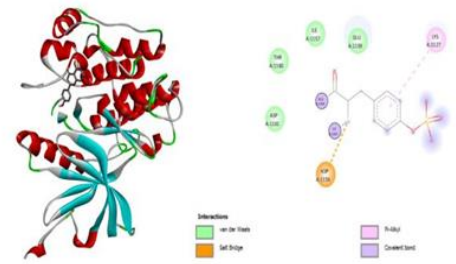


Figure 5E. Effects of Apigenin on PI3K mRNA in High Glucose Induced-adipocyte Cells

Molecular Docking Analysis of Apigenin with Insulin Target Molecules (IR, Akt, IRS-1, PI3K, Glut-4)

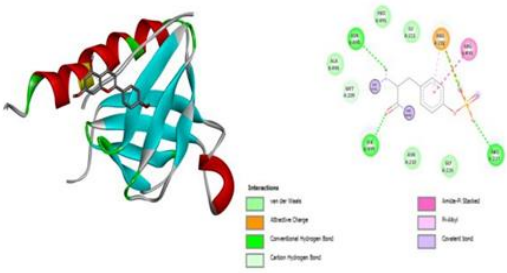
The 3D structures were visualized using Biovia Discovery Studio, showcasing potential interactions and binding affinities of apigenin with these targets. Further, the docking results also show that the phytochemical compound has a high binding affinity between the inflammatory targets as shown below in table-5 & Figures 6-9.

Fig.6



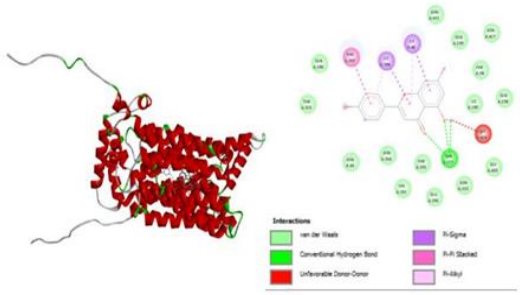
Molecular docking analysis of Apigenin ligand with target IR using PyRx software and 3D &2D structure visualized using Biovia Discovery Studio

Fig.7



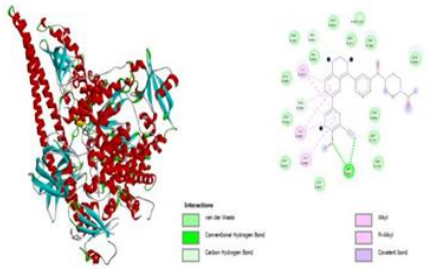
Molecular docking analysis of Apigenin ligand with target IRS1 using PyRx software and 3D &2D structure visualized using Biovia Discovery Studio

Fig.8



Molecular docking analysis of Apigenin ligand with target GLUT4 using PyRx software and 3D &2D structure visualized using Biovia Discovery Studio

Fig.9



Molecular docking analysis of Apigenin ligand with target PI3K using PyRx software and 3D &2D structure visualized using Biovia Discovery Studio

Figure 6-9. Molecular Interaction of Apigenim with Insulin Receptor (Fig.6), IRS-1 (Fig.7), GLUT4 (Fig.8) and PI3K (Fig.9) Target Proteins

Table 5. Illustrate the Molecular Docking Analysis of Apigenim Ligand with Insulin Signaling Pathway Targets

S. No	Ligand	Protein	Binding energy (kcal/mol)
1	Apigenin (5280443)	IR	-7.1
2		IRS1	-6.3
3		GLUT4	-8.8
4		Akt	-7.5
5		PI3K	-8.8

Pharmacokinetic Properties of Apigenin

Lipinski's Rule of Five serves as a crucial guideline for evaluating the drug-likeness of chemical compounds, such as apigenin, particularly in assessing their potential as orally active drugs in humans. In our investigation, apigenin does not exhibited any violation concerning molecular mass, hydrogen donors, and acceptors among the five criteria outlined by Lipinski. The Lipinski violations of apigenin are illustrated in Table 6. The Topological Polar Surface Area (TPSA) plays a pivotal role in predicting the transport properties of drug candidates within the intestines and across the blood-brain barrier (BBB). Moreover, apigenin, with a TPSA score of 90.9, demonstrated a significantly high TPSA value, indicating its preference for a hydrophilic nature. These findings highlight the potential

challenges associated with apigenin's drug-likeness, emphasizing the importance of considering factors like TPSA and Lipinski's rule in the drug development process. Further refinement and optimization may be required to enhance apigenin's pharmacokinetic properties and overall suitability as a drug.

Table 6. ADME Property of Apigenin

Molecule	**Apigenin**	**Ali Log S**	**-4.59**
Formula	$C_{15}H_{10}O_5$	Ali Solubility (mg/ml)	6.88E-03
MW	270.24	Ali Solubility (mol/l)	2.55E-05
#Heavy atoms	20	Ali Class	Moderately soluble
#Aromatic heavy atoms	16	Silicos-IT LogSw	-4.4
Fraction Csp3	0	Silicos-IT Solubility (mg/ml)	1.07E-02
#Rotatable bonds	1	Silicos-IT Solubility (mol/l)	3.94E-05
#H-bond acceptors	5	Silicos-IT class	Moderately soluble
#H-bond donors	3	GI absorption	High
MR	73.99	BBB permeant	No
TPSA	90.9	Pgp substrate	No
iLOGP	1.89	CYP1A2 inhibitor	Yes
XLOGP3	3.02	CYP2C19 inhibitor	No
WLOGP	2.58	CYP2C9 inhibitor	No
MLOGP	0.52	CYP2D6 inhibitor	Yes
Silicos-IT Log P	2.52	CYP3A4 inhibitor	Yes
Consensus Log P	2.11	log Kp (cm/s)	-5.8
ESOL Log S	-3.94	Lipinski #violations	0
ESOL Solubility (mg/ml)	3.07E-02	Ghose #violations	0
ESOL Solubility (mol/l)	1.14E-04	Veber #violations	0
ESOL Class	Soluble	**Egan #violations**	0
Bioavailability Score	0.55	**Muegge #violations**	0
PAINS #alerts	0	**Leadlikeness #violations**	0
Brenk #alerts	0	**Synthetic Accessibility**	2.96

Discussion

Molecular docking is an essential approach in molecular biology and drug development that helps to clarify the interactions between tiny molecules (called ligands) and target proteins (called receptors), which in turn helps to design new therapeutic medicines. Molecular docking is discussed along with its use in rational drug design, difficulties it encounters, effects on lead optimization, and function in comprehending structure-activity correlations.

First of all, by making it easier to screen through huge chemical libraries in search of possible lead compounds that bind to a target protein with high affinity and specificity, molecular docking plays a crucial role in rational drug design. Molecular docking expedites drug discovery and lowers the expense and duration of experimental screening

techniques by forecasting the binding mechanism and affinity of ligands to target proteins. Moreover, molecular docking makes it possible to investigate binding interactions at the atomic level, which offers insightful knowledge about the molecular processes underpinning interactions between drugs and targets [10-14].

Molecular docking is not without its difficulties, though. Since current scoring methods frequently struggle to appropriately score binding poses based on their expected affinities, one significant difficulty is precisely estimating the binding affinity of ligands to target proteins. Furthermore, precise three-dimensional structures of the ligand and the target protein are necessary for molecular docking; however, these structures are not always easily accessible, especially in the case of membrane proteins or protein complexes [15-18]. In spite of these difficulties, lead optimization relies heavily on molecular docking, which directs the alterations and enhancements of lead compounds to enhance their pharmacokinetic, binding, and selectivity characteristics. Through repetition by inserting altered ligands into the target protein's binding site and examining the ensuing binding positions, scientists can find structural alterations that maximize the effectiveness and potency of lead compounds while reducing toxicity and off-target consequences [19-23].

All things considered, molecular docking is a crucial tool in the drug development process, providing insightful information on ligand-receptor interactions and supporting the logical design of new therapeutic medicines. The creation of safe and efficient medications for a variety of disorders is anticipated to be accelerated by molecular docking as computational techniques and scoring functions continue to progress.

Conclusion

In summary, apigenin is a naturally occurring flavonoid component that may be found in large quantities in a variety of fruits, vegetables, teas, and herbs. It has some promise as a medicinal agent for controlling diabetes mellitus and decreasing hyperglycemia. Apigenin exhibits effectiveness in glycemic management and reducing the problems linked to diabetes because to its diverse modes of action, which include boosting insulin sensitivity, encouraging glucose absorption, and exhibiting antioxidant and anti-inflammatory actions. Research indicates that apigenin enhances insulin signaling and glucose absorption in insulin-sensitive tissues by targeting important signaling pathways involved in glucose metabolism, such as the PI3K/AKT pathway, both *in vitro* and *in vivo*. Furthermore, the antioxidant qualities of apigenin aid in shielding pancreatic beta cells from damage brought on by oxidative stress, maintaining both their functionality and ability to secrete insulin. Moreover, apigenin inhibits the activity of enzymes that break down carbohydrates. Moreover, apigenin inhibits the activity of enzymes that break down carbohydrates, including alpha-amylase and alpha-glucosidase, which lowers postprandial glucose levels. These results highlight apigenin's potential as an adjuvant therapy for diabetes control, providing a healthy and natural alternative to current treatment plans. Overall, the evidence to date indicates that apigenin is a natural flavonoid with promise as a valuable therapeutic agent in the fight against diabetes and its complications, even though more research, including clinical trials, is necessary to fully elucidate the therapeutic potential and safety profile of apigenin in the management of hyperglycemia and diabetes.

Conflict of Interest

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

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