Research Article

Rutin and Curcumin as Promising Agents for Diabetes-Associated Neurological Complications: A Molecular Docking and In Vitro Evaluation

Kalpna Kashyap[1], Vivek Chauhan[1], Vineet Mehta[2*]

[1]Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan-333001, India

Email: kalpnakashyap87@gmail.com (Kalpna Kashyap)

Email: pharmvivek16@gmail.com (Dr. Vivek Chauhan)

[2*]Department of Pharmaceutics, Govt. College of Pharmacy, Rohru, District Shimla, Himachal Pradesh-171207, India.

Email: vineet.mehta20@gmail.com

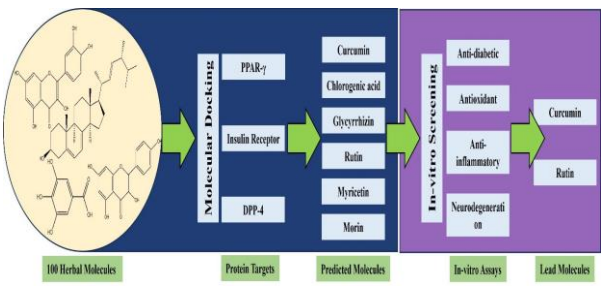
(Corresponding Author)

Abstract

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycaemia, resulting from insulin resistance or relative peripheral insulin deficiency. Hyperglycaemia is associated with inflammatory stress, oxidative stress, and neurodegeneration, which leads to several CNS complications associated with DM. Currently available therapeutics for the management of DM focus on displacing glucose from the blood without affecting the underlying cause of DM or pathological pathways. This study aimed to investigate the antidiabetic potential of herbal molecules and their effect on pathways associated with neurological complications associated with DM. Molecular docking screening of 100 herbal molecules against peroxisome proliferator-activated receptor-γ (PPAR-γ), insulin receptor (IR), and Dipeptidyl Peptidase-4 (DPP4) predicted curcumin, chlorogenic acid, glycyrrhizin, rutin, myricetin, and morin to be the most promising molecules. These molecules were subjected to *in vitro* screening employing antioxidant, anti-inflammatory, neurodegeneration, and antidiabetic protocols. Our results demonstrated rutin and curcumin to be the most promising molecules as these molecules not only demonstrated good antidiabetic potential in *in-silico* studies (docking interaction with DM target proteins), but also demonstrated excellent *in-vitro* antidiabetic activity by inhibiting α-amylase and enhancing 2NBDG uptake. Moreover, these molecules exhibited excellent antioxidant, anti-inflammatory, and neuroprotective potential, suggesting their potential beneficial effects during DM associate CNS complications. In conclusion, rutin and curcumin could be beneficial in the management of DM and associated neurological complications, however, the results need further validation through *in vivo* experimentations.

Keywords: Diabetes mellitus, Molecular docking, Antioxidant, Curcumin, Rutin.

How to cite this article: Kashyap K, Chauhan V, Mehta V, Rutin and Curcumin as Promising Agents for Diabetes-Associated Neurological Complications: A Molecular Docking and In Vitro Evaluation. Int J Drug Deliv Technol. 2026;16(4s): 698-709; DOI: 10.25258/ijddt.16.4s.82



Graphical Abstract

Introduction

Diabetes Mellitus (DM) is a systemic endocrine disorder characterised by elevated blood-glucose levels (hyperglycaemia). Type-I DM occurs due to the autoimmune destruction of insulin-producing pancreatic β-cells, resulting in insufficient peripheral insulin levels. Type-II DM is characterized by decreased insulin sensitivity in the target cells, leading to less or no glucose uptake in the cells.[1,2] According to the International Diabetes Federation (IDF), approximately 589 million adults (11.1% of the global population aged 20-79 years) were living with diabetes in 2024, and is expected to increase to 853 million by 2050.

Mortality and morbidity associated with DM are attributed to the secondary complications associated

with it, which include neuropathy, nephropathy, retinopathy, etc. Hyperglycaemia triggers a pathological condition characterised by increased production of reactive oxygen species (ROS) from both mitochondrial and non-mitochondrial sources. This condition results from the activation of several pro-oxidative signalling pathways, including the hexosamine pathway, polyol pathway, protein-kinase C (PKC) isoforms, and the formation of advanced glycation end-products (AGE). The excessive ROS contributes to resistance mechanisms and diabetes associated CNS complications.[3] Moreover, several reports suggest that prolonged hyperglycemia inflicts neuroinflammation, which, in combination with the enhanced oxidative stress, leads to the development of neurodegeneration and other CNS complications.[4] Therefore, compounds with hypoglycaemic, antioxidant, and anti-inflammatory properties could offer a promising therapeutic strategy for the management of DM.

Currently, several synthetic drugs are used to manage diabetes that primarily focus on reducing postprandial hyperglycaemia. Various drugs, such as AMP-4 activators like metformin, peroxisome proliferator-activated receptor-γ (PPAR-γ) activators like pioglitazone, K-ATPase blockers like gliclazide, and dipeptidyl peptidase-4 (DPP-4) inhibitors like sitagliptin, are a few examples of successful antidiabetic medications. Although these therapies lower the blood glucose level, they do not affect underlying CNS complications and pathological pathways associated with them.[4,5] When compared to synthetic drugs, plant-based molecules show less to no toxicity and have fewer adverse effects. Traditional medicinal herbs with antidiabetic properties can serve as valuable sources for the development of safer hypoglycaemic agents.[6,7] Plants are widely recognised as reliable sources of therapeutic compounds, with numerous synthetic medications derived directly or indirectly from them.[8] They serve as a viable source of bioactive compounds, including alkaloids, glycosides, flavonoids, and polyphenols, which primarily exhibit antidiabetic effects through their anti-inflammatory and antioxidant properties.[9-10] This study aims to identify herbal molecules that could be beneficial in the management of DM and associated neural complications using *in silico* and *in vitro* approaches.

Material and Methods:

Selection of herbal molecules:

A total of 100 herbal molecules were selected from the literature that have shown potential to target various pathways associated with DM and CNS complications. These pathways include oxidative stress, neuroinflammation, and neurodegeneration. Selected heral molecules were subjected to molecular docking against several targets of DM, including PPAR-γ, insulin receptor (IR), and DPP-4.

Preparation of Ligands and Receptors for Molecular Docking

The 2D structures of the selected herbal molecules were generated using ChemDraw Ultra 7.0 and converted to 3D structures using the MarvinSketch tool. The AutoDock Vina 1.5.6 was used to convert the saved 3D structure of ligands from .pdb to .pdbqt format and saved. Similarly, the structures of the standard drugs were prepared and saved in .pdbqt format.[4] The crystallized structures of the proteins, PPAR-γ (5YCP), IR (1IR3), and DPP-4 (IPFQ) receptors, were obtained from the Protein Data Bank, having resolutions of 2.7, 2.5, and 1.9 Å, respectively. AutoDock Vina was used to prepare the selected protein for docking by removing unwanted residues and water molecules, adding polar hydrogens and charges, and saving it in .pdbqt format for analysis.

Molecular Docking

Molecular docking was performed using Auto Dock Tools-1.5.6 software, where the prepared ligands and standard drugs were docked with the selected target proteins. The grid box was configured to determine the coordinates of the proteins. The grid box was centred at x = 25.378, y = 11.101, and z = 31.782, with dimensions of 60 for x, y, and z. Auto-grid and auto-dock widgets were used to prepare docking and grid files. Docking was performed, and the results were reported in terms of free energy (kcal/mol). The 2D interactions of the docked results were visualized using Discovery Studio software.[4,11,12]

***In-Vitro* Evaluation**

Various *in vitro* assays were performed to further validate the docking results. The best compounds predicted from the docking analysis on the basis of binding affinities were subjected to *in vitro* assays to predict the potential of these molecules to target DM, oxidative stress, neuroinflammation, and neurodegeneration.

Anti-diabetic activity

α-Amylase inhibitory activity

The ability of test substances to inhibit α-amylase activity was evaluated using the method described by Ali et al. with slight modifications.[13] 30 µl of test compounds at various concentrations (50-500 µM) were mixed with 60 µl of enzyme (1U/ml prepared in PBS; pH-6.9) and incubated for 10 minutes at 37ºC in

95-well plates. An equal volume of pure water was added in place of the test compounds in the enzyme control reaction. Further, the reaction was terminated by adding 30 µl HCl (1M) and 120 µl of 5% w/v IKI. Absorbance was recorded at 630 nm using a UV spectrophotometer as a measure of enzyme activity. All experiments were conducted in triplicate. Percentage α-amylase inhibition was calculated using the following equation, and the IC_{50} values were determined.

% amylase inhibition = 1 – (Absorbance of test/ Absorbance of control) × 100

NBDG uptake assay

The effect of herbal molecules on glucose uptake was determined by using a fluorescently labelled 2-NBDG glucose analogue as per the method described by Jung et al.[14] The glucose absorption activity of the test compounds was assessed in L6 cells using 96-well clear-bottom black fluorescence plates. Cell cultures at 70-80% confluence were used for the final test, where cells were serum-starved overnight, cells were incubated in HEPES-buffered Krebs Ringer Phosphate Solution (KRP buffer) containing 0.1% BSA for 30 minutes at 37°C. Cells were treated with different concentrations of the test sample (0.625-5 µM) in glucose-free culture media at 37°C. Further, 2-NBDG was added to the culture plates at a final concentration of 200 µg/mL. At the end of the treatment, the supernatant was aspirated, and 100 µL PBS was added to each well. An inverted fluorescent microscope was used to detect the 2-NBDG uptake by cells through fluorescence reading, and the results were measured as corrected total cell fluorescence (CTCF).

Antioxidant Assay

DPPH Radical Scavenging Assay

The antioxidant capabilities of the identified ligands were assessed through UV-spectrophotometric analysis using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This assay was performed as per the procedures described by Mehta et al. and Chen et al., with slight modifications.[15,16] To prepare the working concentrations for the DPPH inhibition assays, a stock solution (100 µM) of each test compound was prepared in 95% methanol and subsequently diluted to concentrations ranging from 0.625-5 µM. 3 ml DPPH solution (0.4 mM prepared in 95% methanol) was mixed with 1 ml solution of different herbal molecules (test), ascorbic acid (standard), or 95% methanol (blank). The reaction mixture was mixed thoroughly and incubated in the dark at 37 °C for 30 minutes. The entire experiment was conducted in triplicate, and the absorbance of the reaction mixtures was recorded using a UV spectrophotometer at 517 nm. DPPH radical scavenging activity was calculated by using the following equation, and the IC_{50} values were determined.

%DPPH radical scavenging =1- (Absorbance of test/ Absorbance of control) × 100

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the herbal molecules was evaluated using a modified version of the method described by Wang et al. and Luo et al.[17,18] Herbal molecules at concentrations of 0.625, 1.25, 2.5, and 5 µM were incubated with 2 mM EDTA–Fe (0.5 ml), 3% H_2O_2 (1.0 ml), and 0.360 mg/ml crocus in 4.5 ml sodium phosphate buffer (pH 7.4) for 30 min at 37°C. The detection of hydroxyl radicals was performed by measuring the absorbance at 520 nm using a UV spectrophotometer. The entire experiment was conducted in triplicate. The hydroxyl radical-scavenging activity was calculated by using the following equation, and the IC_{50} values were determined.

% Hydroxyl radical scavenging =1- (Absorbance of test/ Absorbance of control) × 100

***In-vitro* Neurotoxicity Assay**

The impact of herbal molecules on $HgCl_2$-induced neurotoxicity was assessed using Neuro2A cell lines. The evaluation employed the MTT assay as described previously by Nagu et al.[4] EMEM media was used to culture Neuro2A cells until they reached 70% confluency. Subsequently, the growth media were replaced with serum-free EMEM media. To this, 10 µl solution (300 µM) of each herbal molecule was added. An equivalent volume of PBS (pH 7.4) was utilized for the control reaction. The culture plates were incubated for 6 h in a humidified CO_2 incubator at 37ºC. Thereafter, 25 µM $HgCl_2$ was transferred to each cell to induce neurotoxicity, and culture plates were incubated for 24 hours in a CO_2 incubator at 37ºC. The culture media was removed, cultures were washed with PBS, and cell lysis was done by adding DMSO. Absorption of the formazan was measured spectrophotometrically at 570 nm as a measure of cell viability. The entire experiment was conducted in triplicate, and the percentage of neuroprotection was calculated.

Anti-inflammatory Assay

Inhibition of protein denaturation

In vitro anti-inflammatory properties of natural compounds were assessed by examining the ability of herbal molecules to prevent albumin denaturation as per the method outlined by Ullah et al., with some

modifications.[19] The reaction mixture consisted of 1 ml of herbal compounds (0.625-5 µM; test) or diclofenac sodium (0.625-5 µM) (standard) or distilled water (as a blank) and 1 ml of 1% aqueous bovine serum albumin prepared in PBS (pH 6.4). These mixtures were incubated at 37°C for 30 minutes. This was followed by a denaturing temperature of 57°C for 5 minutes. Each tube was subsequently cooled under running tap water, and the absorbance was measured spectrophotometrically at 660 nm. The entire experiment was conducted in triplicate. Percentage inhibition of protein denaturation was calculated using the following equation, and IC_{50} values were determined.

Percentage inhibition = (Absorbance of control – Absorbance of test)/ Absorbance of control) × 100

Inhibition of heat-induced haemolysis

Heat-induced haemolysis is another *in vitro* model to determine the anti-inflammatory potential of test molecules.[20,21] Reaction mixture consisted of 5 ml isotonic buffer having 1 mL of different concentrations (0.625-5 µM) of test molecules and 50 µL of erythrocyte suspension. An equal volume of vehicle was added to the isotonic buffer in the control reaction. Reaction mixtures were incubated at 54 °C for 20 minutes over a water bath, followed by centrifugation at 1300 g for 3 minutes. Absorbance of the resulting supernatant was measured at 540 nm. The percentage inhibition of haemolysis was estimated by using the following equation, and IC_{50} values were determined.

%Inhibition of hemolysis = (Absorbance of control – Absorbance of test)/ Absorbance of control) × 100

Statistical Analysis

GraphPad Prism 6 software was utilized to evaluate statistical significance. The results were presented as mean ± SD. To determine statistical significance, a one-way ANOVA followed by Dunnett's multiple comparison post hoc test was employed (* $p < 0.05$, $p < 0.01$, and * $p < 0.001$) and Duncan's multiple range test at $p < 0.05$.

Results and Discussion

Molecular Docking Study

A total of 100 herbal molecules were subjected to molecular docking screening against PPAR-γ, IR, and DPP4 using Auto Dock tools. The study employed rosiglitazone, insulin, and sitagliptin as internal standards for PPAR-γ (5YCP), IR (1IR3), and DPP4 (1PFQ), respectively. The molecules were screened based on their binding affinity and the amino acids involved in the ligand-receptor interaction. Our results predicted baohuside, cyanidin-3-rhamnoside, curcumin, chlorogenic acid, desmanthin, guaijaverin, glycyrrhizin, myricetin, morin, and rutin as the most promising herbal molecules. The results of docking interactions with PPAR-γ are detailed in Table 1.

Table 1: Docking interactions of lead molecules with PPAR-γ (5YCP) and amino acids involved in the binding interaction.

S. No.	Molecules	Docking Score (kcal/mol)	Common Interacting Amino Acids
1.	Rosiglitazone (Standard)	-7.6	ARG288, CYS285, GLN286, GLU259, GLY284, HIS449, LEU330, LYS37, PHE287, SER289, SER342
2.	Baohuside	-8.9	GLU259, GLY284, SER 342
3.	Cyanidin-3-rhamnoside	-8.9	ARG288, CYS285, LEU255, PHE287
4.	Curcumin	-7.1	ARG288, CYS285, GLY284, HIS449, LEU330, PHE287, SER289, SER342
5.	Chlorogenic acid	-7.1	GLU259, GLY284, SER342
6.	Desmanthin1	-10.3	ARG280, ARG288, ILE249, ILE 341, GLY284, PHE287, SER342
7.	Guaijaverin	-8.5	ARG288, LEU255, PHE287, SER342
8.	Glycyrrhizin	-9.8	GLY284, PHE287, SER342

9.	Myricetin	-7.6	ARG288 LEU255, SER342
10.	Morin	-6.9	GLU259, LEU255, SER342
11.	Rutin	-8.1	ARG288, GLY284, PHE287, PHE287, LEU330, SER289, SER342

The docking analysis indicates that desmanthin and glycyrrhizin exhibited the lowest binding energy of −10.3 kcal/mol and −9.8 kcal/mol, respectively, suggesting their potential to influence PPAR-γ activity. The docking score for the standard drug, rosiglitazone, was observed to be −7.6 kcal/mol, which was comparable to curcumin (−7.1 kcal/mol) and chlorogenic acid (−7.1 kcal/mol). Standard drugs demonstrated strong interaction with ARG288, CYS285, GLN286, GLU259, GLY284, HIS449, LEU330, LYS37, PHE287, SER289, and SER342. Notably, the binding energy of rutin with the target receptor was −8.1 kcal/mol and demonstrated the highest amino-acid interactions with the receptor, indicating its strong potential to modulate receptor activity. The RMSD values for the herbal molecules ranged from 0.00 to 1.0, which were comparable to rosiglitazone, indicating good interaction.

Table 2 depicts the docking interaction results for IR (1IR3) and test molecules. Our findings indicate that amino acids such as ALA1028, ASN1137, ASP1083, ASP1150, GLN1004, GLY1003, GLY1005, and GLY1082 play a significant role in the interaction of insulin with IR. Molecules interacting with these amino acids are predicted to effectively target IR. Morin and rutin yield the most favourable outcomes with docking scores of −9.00 kcal/mol and −10.1 kcal/mol, respectively. The binding interactions of these molecules surpassed the standard, which had a docking score of −6.9 kcal/mol. Further, rutin and curcumin showed strong interactions with amino acids like ALA1028, ASP1083, ASP1150, GLN1004, LYS1030, MET1079, MET1139, and VAL1010. Docking interaction of other herbal molecules was observed in the range of −7.0 kcal/mol to -8.8 kcal/mol, which was comparable to the standard drug. The RMSD values for the herbal molecules ranged from 0.00 to 0.94, indicating good interaction and suggesting their potential use on the insulin receptor.

DPP4 (1PFQ) is a key enzyme involved in glucose metabolism, and the docking interactions of the test molecules as 1PFQ are depicted in Table 3. Our results suggest that ALA210, ALA213, ASP302, LEU214, PHE208, PRO159, SER158, SER212, THR156, TRP154, TRP157, TRP115, and TRP216 are the primary amino acids involved in the interaction between sitagliptin and the protein. Our findings identified glycyrrhizin as the most effective ligand for DPP4 with a docking score of -10.3 kcal/mol. The docking scores of other herbal compounds indicated superior interaction (-7.4 kcal/mol to -9.4 kcal/mol) with DPP4 when compared to Sitagliptin (-6.9 kcal/mol). The RMSD values for the herbal compounds ranged from 0.00 to 1.0, comparable to sitagliptin, indicating a strong interaction.

Table 2: Docking interactions of lead molecules with IR (1IR3) and amino acids involved in the binding interaction.

S. No.	Molecules	Docking Score (kcal/mol)	Common Interacting Amino Acids
1.	Insulin (Standard)	-6.9	ALA1028, ASP1083, ASP1150, GLN1004, LYS1030, MET1079, MET1139, VAL1010
2.	Baohuside	-7.3	ASP1083, ASP1150, LEU1002, MET1076, MET1139, VAL1010
3.	Cyanidin-3-rhamnoside	-8.8	ASP1083, GLN1004, GLY1003, LEU1002, LYS1030, VAL1010
4.	Curcumin	-7.0	ASP1083, ASP1150, GLN1004, LYS1030, MET1079, MET1139, VAL1010
5.	Chlorogenic acid	-8.5	ASP1083, GLN1004

6.	Desmanthin1	-8.2	GLY1005, LYS1030, MET1139, SER1006, VAL1010
7.	Guaijaverin	-8.9	GLY1082, LEU1002, LYS1030, MET1079, VAL1010
8.	Glycyrrhizin	-7.9	ASP1083, GLN1004, MET1076, MET1139
9.	Myricetin	-8.5	LEU1002, LYS1030, MET1139, VAL1010
10.	Morin	-9.0	GLY1082, LEU1002, LYS1030, MET1139, VAL1010
11.	Rutin	-10.1	ALA1028, ASP1083, GLY1003, LEU1002, LYS1030, MET1139, VAL1010

Table 3 Docking interactions of lead molecules with DPP4 (1PFQ) and amino acids involved in the binding interaction.

S. No.	Molecules	Docking Score (kcal/mol)	Interacting Amino Acids
1.	Sitagliptin (Standard)	-6.9	ALA210, ALA213, ASP302, LEU214, PHE208, PRO159, SER158, SER212, THR156, TRP154, TRP157, TRP115, TRP216
2.	Baohuside	-8.5	LEU214, PRO159, SER158, THR156, TRP154, TRP157, TRP215, TRP216
3.	Cyanidin-3-rhamnoside	-8.5	PRO159, SER158, THR156, TRP157, TRP216
4.	Curcumin	-8.6	THR156, TRP154, TRP157, TYR120, TYR128, VAL155
5.	Chlorogenic acid	-7.4	THR156, TRP154, TRP157
6.	Desmanthin 1	-9.4	LEU214, PRO159, THR156TRP157, TRP21, TRP216
7.	Guaijaverin	-8.4	PRO159, THR156 TRP157, TRP216
8.	Glycyrrhizin	-10.3	ALA210, LEU214, PHE208, SER212, THR156 TRP154, TRP157, TRP215, TRP216
9.	Myricetin	-8.2	PRO159, THR156, TRP154, TRP157
10.	Morin	-7.8	PRO159, THR156, TRP154, TRP157
11.	Rutin	-8.3	THR156, TRP154, TRP157

We analysed the screening results of docking analysis based on the binding affinity, amino acids involved in the interaction, bond length, and type of bonds. Out of

100 screened and 10 most promising molecules to target DM through PPAR-γ, IR, and DPP-4 pathways, we selected curcumin, chlorogenic acid, glycyrrhizin, rutin, myricetin, and morin as the lead molecules for further *in vitro* analysis.

Antidiabetic Assay

α-amylase is an enzyme that is responsible for the conversion of complex polysaccharides into simpler and absorbable monomeric units (D-glucose), thereby increasing blood sugar levels. Inhibiting this enzyme is considered a good approach to control post-prandial blood sugar spikes during DM. There is numerous scientific evidences where inhibition of α-amylase by the test molecules has been demonstrated to be promising anti diabetic candidates.[22-24] In the present study, we determined the inhibitory effect of curcumin, chlorogenic acid, glycyrrhizin, rutin, myricetin, and morin on α-amylase activity in terms of IC_{50} values (Figure 1).

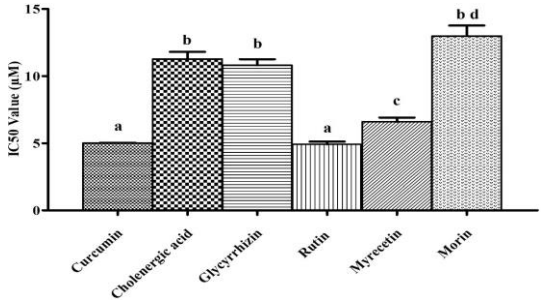


Fig. 1: The effect of herbal molecules on α-amylase activity in terms of IC_{50} values. Results are depicted as mean ± SD (n = 3) of the observed IC_{50} values. Different letters above the error bar represent that the results are significantly different at p < 0.05 by Duncan's multiple range test.

Our results suggest that rutin is the most potent molecule to inhibit α-amylase activity, as indicated by the lowest IC_{50} value. Moreover, curcumin and myricetin also demonstrated promising results in inhibiting α-amylase activity. These results suggest that rutin, curcumin, and myricetin could be beneficial in the management of DM by controlling post-prandial blood glucose levels by inhibiting α-amylase activity.

The glucose uptake assay is another *in vitro* assay to screen molecules with potential antidiabetic properties. This method involves the use of fluorescent glucose, 2-NBDG, which is absorbed through GLUT transporters. Due to its fluorescent nature, its accumulation within cells is quantified using a fluorescence microscope as a marker of glucose uptake.[25] We determined the effect of curcumin, chlorogenic acid, glycyrrhizin, rutin, myricetin, and morin on glucose uptake in L6 cells and compared the results with insulin serving as a positive control. The results are depicted in Figure 2 in terms of corrected total cell fluorescence (CTCF), where the higher CTCF value suggests increased glucose uptake and vice versa.

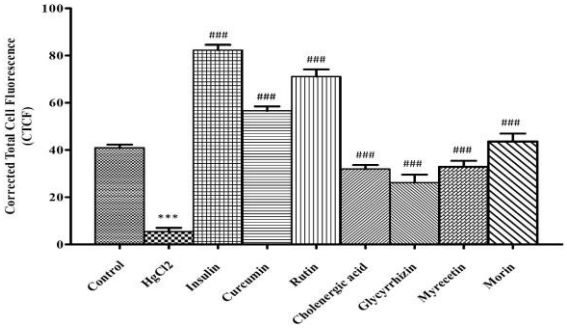


Fig. 2: Effect of herbal molecules on 2NBDG uptake in the Neuro2A cell lines. Results are depicted as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple comparison post hoc test at ***p <0.001, **p < 0.01, and *p <0.05 (control vs treatments).

Our results demonstrated the highest CTCF value for insulin that was significantly ($p < 0.001$) higher than the control. Rutin and curcumin demonstrated significant ($p < 0.001$) improvement in 2NBDG uptake as indicated by the higher CTCF value than control. Results observed for rutin and curcumin were promising and were comparable to insulin. However, chlorogenic acid, glycyrrhizin, myricetin, and morin failed to improve 2NBDG uptake, and the results were comparable to the control. These findings suggest that rutin and curcumin could be promising drug molecules for the management of DM, which could enhance glucose uptake during DM. These findings are consistent with the literature reports where molecules capable of enhancing 2NBDG uptake have been demonstrated to be promising anti-diabetic candidates.[26,27]

Antioxidant Assay

DPPH radical scavenging and hydroxyl radical scavenging assays are the most widely used methods to determine the antioxidant activity of herbal and synthetic molecules. In solution, DPPH is a stable free radical having a deep violet colour. When an antioxidant molecule is introduced to the DPPH solution, it reduces the DPPH molecules, resulting in a colour change to yellow. This colour change is determined spectrophotometrically (517 nm) as a measure of the antioxidant potential of the test molecules.[28] Likewise, the hydroxyl radical scavenging assay is based on the potential of test compounds to neutralize hydroxyl radicals, which are

generated in the reaction mixture through the Fenton reaction.[29,30] Results of the *in vitro* antioxidant activity are depicted in Figure 3.

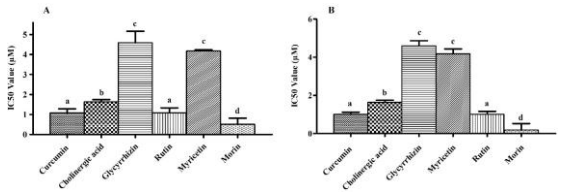


Fig. 3: The effect of herbal molecules on *in-vitro* DPPH radical scavenging assay (A) and hydroxyl radical scavenging assay (B). Results are depicted as mean ± SD (n = 3) of the observed IC_{50} values. Different letters above the error bar represent that the results are significantly different at $p < 0.05$ by Duncan's multiple range test.

Our results demonstrated curcumin, morin, and rutin as the most potent molecules in scavenging DPPH free radicals and hydroxyl radicals. Literature suggests that DM is associated with enhanced oxidative stress in the CNS that leads to several complications, amongst which neurodegeneration,[31] cognitive impairment,[32,33] dementia,[34] and depression[35] are the most prevalent.

Our results suggest that curcumin, morin, and rutin have significant antioxidant activity, as indicated by the lower IC_{50} values. These molecules could be beneficial in attenuating oxidative stress-mediated complications associated with DM. Literature suggests that curcumin relieves oxidative stress, which is responsible for the macrovascular and microvascular complications associated with DM, and attenuates the insulin secretion, glucose transport pathway, DNA damage, vascular permeability, and generation of free fatty acids contributing to endothelial dysfunctions.[36] Likewise, our results for morin and rutin are also in line with the existing literature that suggests that these molecules have significant antioxidant potential that is responsible for their beneficial effects during oxidative stress.[37,38]

Anti-inflammatory Assay

Anti-inflammatory potential of curcumin, chlorogenic acid, glycyrrhizin, rutin, myricetin, and morin was assessed by two *in-vitro* anti-inflammatory assays, inhibition of protein denaturation and inhibition of heat-induced hemolysis. Inhibition of protein denaturation assay is based on the ability of the test compound to attenuate structural damage to the protein structure, caused by physical or chemical stress, which is associated with inflammatory conditions. Denaturation of proteins leads to the formation of auto-antigen and contributes to inflammation-mediated complications.[39] Likewise, inhibition of heat-induced hemolysis is another *in vitro* method to screen the anti-inflammatory potential of test molecules. This assay is based on determining the potential of the test substance to stabilize the plasma membrane of red blood cells (RBCs) when exposed to elevated temperatures, thereby preventing or reducing hemolysis. Heat exposure destabilizes and disrupts RBC membranes, mimicking the lysis of lysosomal membranes during inflammation.[40]Results of the *in vitro* anti-inflammatory activity are depicted in Figure 4.

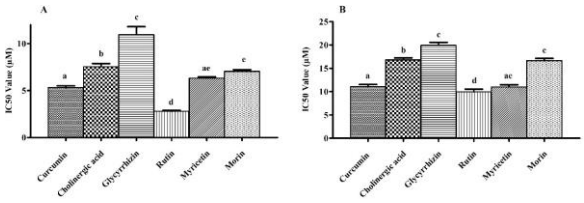


Fig. 4: The effect of herbal molecules on *in vitro* inhibition of protein denaturation (A) and inhibition of heat-induced hemolysis (B). Results are depicted as mean ± SD (n = 3) of the observed IC_{50} values. Different letters above the error bar represent that the results are significantly different at $p < 0.05$ by Duncan's multiple range test.

Our results demonstrated rutin to be the most potent anti-inflammatory molecule, having the lowest IC_{50} value in both studies. Moreover, curcumin and myricetin also demonstrated good anti-inflammatory potential, but had higher IC_{50} values than rutin. Literature suggests that DM is associated with enhanced inflammatory stress in the CNS that leads to several complications, amongst which neurodegeneration,[41] cognitive impairment,[32,42], dementia,[42] and depression[43] are the most prevalent. Our results suggest that rutin, curcumin, and myricetin have significant anti-inflammatory activity, as indicated by the lower IC_{50} values. These molecules could be beneficial in attenuating inflammatory stress-mediated complications associated with DM. Our findings are consistent with the recent literature reports where rutin has been demonstrated to have significant anti-inflammatory potential to attenuate inflammation-mediated complications.[38,44] Likewise, the anti-inflammatory potential of curcumin and myricetin to attenuate various complications has also been reported,[45-47] consolidating our findings

Cell Viability Assay

$HgCl_2$ is a recognized neurotoxin and is known to inflict neuronal damage through apoptosis, necrosis, and cytoskeleton damage. We used $HgCl_2$ to inflict neurodegeneration in Neuro2A cell lines and evaluated the neuroprotective potential of herbal compounds in

terms of percentage of cell viability. Results of the effect of herbal molecules on $HgCl_2$-induced neurodegeneration are depicted in Figure 5.

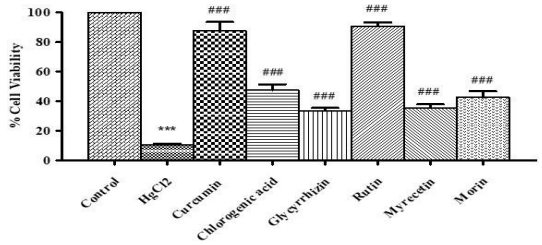


Fig. 5: Effect of herbal molecules on $HgCl_2$-induced neurodegeneration in terms of percent cell viability. Results are depicted as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple comparison post hoc test at $^{\#\#\#\#}p < 0.001$ (control vs $HgCl_2$) and $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$ ($HgCl_2$ vs treatments).

Our results suggest that $HgCl_2$ inflicted significant neurodegeneration, as the cell viability was significantly lower when compared to the control. Treating these cells with herbal molecules improved cell viability, suggesting neuroprotective effects. Our findings suggest that curcumin and rutin are the most promising molecules. Treating Neuro2A cells with curcumin and rutin significantly improved cell viability and attenuated neurodegeneration in a dose-dependent manner. Although improvement in cell viability was also observed for other molecules, the results were not as promising as those observed for curcumin and rutin. Our findings are consistent with the literature, where $HgCl_2$ is known to induce significant neurodegeneration in cell lines.[4] Moreover, literature suggests that herbal molecules capable of inhibiting $HgCl_2$-induced neurodegeneration could be a promising candidate for the management of CNS complications.[4] Our results for curcumin and rutin are in line with the literature, where these molecules have been reported to possess the potential to mitigate neurodegeneration.[48-51] These results suggest that rutin and curcumin could be promising molecules to attenuate DM and associated neurodegeneration.

Conclusion

The present work was focused on identifying herbal molecules that could not only be beneficial for the management of DM, but also could target various pathways associated with the development of DM mediated neurological complications. For this, a combination of molecular docking and an *in vitro* screening approach was followed. Our findings demonstrated rutin, curcumin, and myricetin to be the most promising herbal molecules. These molecules demonstrated good interaction with PPAR-γ, IR, and DPP-4 receptors in docking studies. Moreover, these molecules demonstrated significant antidiabetic activity (inhibited α-amylase and enhanced glucose uptake), promoted neuronal survival, prevented neurodegeneration, and demonstrated excellent antioxidant and anti-inflammatory activities. Based on these findings, we propose that rutin, curcumin, and myricetin could be beneficial in the management of DM and associated complications by targeting glucose homeostasis, neurodegeneration, oxidative stress, and inflammatory stress. However, these findings are preliminary and need further experimental validation through *in vivo* and clinical studies. Based on our findings, we propose rutin, curcumin, and myricetin as promising lead molecules for the management of DM and associated neurological complications.

Acknowledgment

The authors would like to acknowledge the SJJT University, Jhunjhunu, Govt. College of Pharmacy, Rohru, and the Govt. Pharmacy College, Nagrota Bagwan, for providing us with the facility to carry out this research work.

Author's Contribution

KK contributed to study design, performed Docking studies & experiments, VC analyzed results, and prepared the 1st draft of the manuscript.VM contributed to the analysis of the results, provided technical support, supervised experiments, and edited the final draft of the manuscript.

Conflict of Interest

The authors declare no conflict of interest with respect to this article.

References

1. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Physical therapy. 2008;88(11):1322-35.
2. Atlas D. International diabetes federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation. 2015;33(2).
3. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. Journal of hypertension. 2003;21(1):3-12.
4. Nagu P, Pathan AK, Mehta V. Identification of Herbal Molecules for the Treatment of Alzheimer's Disease Through a Combination of Molecular Docking and In-Vitro Analysis. Journal of Scientific & Industrial Research (JSIR). 2023;82(05):504-14.

5. Melnyk JP, Marcone MF. Aphrodisiacs from plant and animal sources—A review of current scientific literature. Food research international. 2011;44(4):840-50.
6. Patel DK, Kumar R, Laloo D, Hemalatha S. Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. Asian Pacific Journal of Tropical Disease. 2012;2(3):239-50.
7. Sunil C, Agastian P, Kumarappan C, Ignacimuthu S. In vitro antioxidant, antidiabetic and antilipidemic activities of Symplocos cochinchinensis (Lour.) S. Moore bark. Food and chemical toxicology. 2012 May 1;50(5):1547-53.
8. Arumugam G, Manjula P, Paari N. A review: Anti diabetic medicinal plants used for diabetes mellitus. Journal of Acute Disease. 2013;2(3):196-200.
9. Li GQ, Kam A, Wong KH, Zhou X, Omar EA, Alqahtani A, Li KM, Razmovski-Naumovski V, Chan K. Herbal medicines for the management of diabetes. Diabetes: An old disease, a new insight. 2012:396-413.
10. Chukwuma CI, Matsabisa MG, Ibrahim MA, Erukainure OL, Chabalala MH, Islam MS. Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: A review. Journal of ethnopharmacology. 2019;235:329-60.
11. El-Hachem N, Haibe-Kains B, Khalil A, Kobeissy FH, Nemer G. AutoDock and AutoDockTools for protein-ligand docking: beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a case study. InNeuroproteomics: Methods and Protocols 2017 (pp. 391-403). New York, NY: Springer New York.
12. Kumar A, Mehta V, Raj U, Varadwaj PK, Udayabanu M, Yennamalli RM, Singh TR. Computational and in-vitro validation of natural molecules as potential acetylcholinesterase inhibitors and neuroprotective agents. Current Alzheimer Research. 2019;16(2):116-27.
13. Ali H, Houghton PJ, Soumyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. Journal of ethnopharmacology. 2006;107(3):449-55.
14. Jung CH, Lee DH, Ahn J, Lee H, Choi WH, Jang YJ, Ha TY. γ-Oryzanol enhances adipocyte differentiation and glucose uptake. Nutrients. 2015;7(6):4851-61.
15. Mehta V, Sharma A, Tanwar S, Malairaman U. In vitro and in silico evaluation of the antidiabetic effect of hydroalcoholic leaf extract of Centella asiatica. International Journal of Pharmacy and Pharmaceutical Sciences. 2016;8(8):357-62.
16. Chen Y, Wang M, Rosen RT, Ho CT. 2, 2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from Polygonum multiflorum thunb. Journal of agricultural and food chemistry. 1999;47(6):2226-8.
17. Wang J, Zhang Q, Zhang Z, Li Z. Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica. International journal of biological macromolecules. 2008;42(2):127-32.
18. Luo A, He X, Zhou S, Fan Y, Luo A, Chun Z. Purification, composition analysis and antioxidant activity of the polysaccharides from Dendrobium nobile Lindl. Carbohydrate Polymers. 2010;79(4):1014-9.
19. Ullah HA, Zaman S, Juhara F, Akter L, Tareq SM, Masum EH, Bhattacharjee R. Evaluation of antinociceptive, in-vivo & in-vitro anti-inflammatory activity of ethanolic extract of Curcuma zedoaria rhizome. BMC complementary and alternative medicine. 2014;14(1):346.
20. Sakat S, Tupe P, Juvekar A. Gastroprotective effect of methanol extract of Oxalis corniculata Linn (whole plant) experimental animals. Planta Medica. 2010;76(12):P090.
21. Raghavendra VB. In vitro anti-inflammatory, lipoxygenase, xanthine oxidase and acetycholinesterase inhibitory activity of Tecoma stans (l.) Juss. Ex kunth. International Journal of Pharma and Bio Sciences. 2011;2(2):275-85.
22. Kashtoh H, Baek KH. New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects. Plants. 2023;12(16):2944.
23. Tolmie M, Bester MJ, Apostolides Z. Inhibition of α-glucosidase and α-amylase by herbal compounds for the treatment of type 2 diabetes: a validation of in silico reverse docking with in vitro enzyme assays. Journal of Diabetes. 2021;13(10):779-91.
24. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. Chemical biology & drug design. 2021;98(4):539-60.
25. Mehta V, Udayabanu M. Investigation of the Effect of Quercetin on Neurological Alterations

During Type 2 Diabetes Mellitus in Swiss Albino Mice (Doctoral dissertation, Jaypee University of Information Technology, Solan, HP) 2019.

26. Majeed S, Danish M, Zakariya NA, Hashim R, Ansari MT, Alkahtani S, Hasnain MS. In Vitro evaluation of antibacterial, antioxidant, and antidiabetic activities and glucose uptake through 2-NBDG by Hep-2 liver cancer cells treated with green synthesized silver nanoparticles. Oxidative Medicine and Cellular Longevity. 2022;2022(1):1646687.
27. Le HL, To DC, Tran MH, Do TT, Nguyen PH. Natural PTP1B inhibitors from Polygonum cuspidatum and their 2-NBDG uptake stimulation. Natural Product Communications. 2020;15(9):1934578X20961201.
28. Alinejad B, Ghorbani A, Sadeghnia HR. Effects of combinations of curcumin, linalool, rutin, safranal, and thymoquinone on glucose/serum deprivation-induced cell death. Avicenna Journal of Phytomedicine. 2013;3(4):321.
29. Giordano A, Morales-Tapia P, Moncada-Basualto M, Pozo-Martínez J, Olea-Azar C, Nesic A, Cabrera-Barjas G. Polyphenolic composition and antioxidant activity (ORAC, EPR and cellular) of different extracts of Argylia radiata vitroplants and natural roots. Molecules. 2022;27(3):610.
30. Liu W, Wang J, Zhang Z, Xu J, Xie Z, Slavin M, Gao X. In vitro and in vivo antioxidant activity of a fructan from the roots of Arctium lappa L. International Journal of Biological Macromolecules. 2014;65:446-53.
31. Mule NK, Singh JN. Diabetes mellitus to neurodegenerative disorders: is oxidative stress fueling the flame?. CNS & Neurological Disorders-Drug Targets-CNS & Neurological Disorders). 2018;17(9):644-53.
32. Damanik J, Yunir E. Type 2 diabetes mellitus and cognitive impairment. Acta Med Indones. 2021;53(2):213-20.
33. Hoyos CM, Colagiuri S, Turner A, Ireland C, Naismith SL, Duffy SL. Brain oxidative stress and cognitive function in older adults with diabetes and pre-diabetes who are at risk for dementia. Diabetes Research and Clinical Practice. 2022;184:109178.
34. Hatanaka H, Hanyu H, Fukasawa R, Sato T, Shimizu S, Sakurai H. Peripheral oxidative stress markers in diabetes-related dementia. Geriatrics & gerontology international. 2016;16(12):1312-8.
35. Réus GZ, Carlessi AS, Silva RH, Ceretta LB, Quevedo J. Relationship of oxidative stress as a link between diabetes mellitus and major depressive disorder. Oxidative medicine and cellular longevity. 2019;2019(1):8637970.
36. Cox FF, Misiou A, Vierkant A, Ale-Agha N, Grandoch M, Haendeler J, Altschmied J. Protective effects of curcumin in cardiovascular diseases—Impact on oxidative stress and mitochondria. Cells. 2022;11(3):342.
37. Cakmak F, Kucukler S, Gur C, Comakli S, Ilerituerk M, Kandemir FM. Morin provides therapeutic effect by attenuating oxidative stress, inflammation, endoplasmic reticulum stress, autophagy, apoptosis, and oxidative DNA damage in testicular toxicity caused by ifosfamide in rats. Iranian Journal of Basic Medical Sciences. 2023;26(10):1227.
38. Ahmed OM, Elkomy MH, Fahim HI, Ashour MB, Naguib IA, Alghamdi BS, Mahmoud HU, Ahmed NA. Rutin and quercetin counter doxorubicin-induced liver toxicity in wistar rats via their modulatory effects on inflammation, oxidative stress, apoptosis, and Nrf2. Oxidative medicine and cellular longevity. 2022;2022(1):2710607.
39. Esho BA, Samuel B, Akinwunmi KF, Oluyemi WM. Membrane stabilization and inhibition of protein denaturation as mechanisms of the anti-inflammatory activity of some plant species. Trends in Pharmaceutical Sciences and Technologies. 2021;7(4):269-78.
40. Sabalingam S. In-vitro approaches to evaluate the anti-inflammatory potential of phytochemicals: A Review. Journal of Drug Delivery & Therapeutics. 2025;15(1):187-92.
41. Starace V, Battista M, Brambati M, Cavalleri M, Bertuzzi F, Amato A, Lattanzio R, Bandello F, Cicinelli MV. The role of inflammation and neurodegeneration in diabetic macular edema. Therapeutic Advances in Ophthalmology. 2021;13:25158414211055963.
42. Ortiz GG, Huerta M, González-Usigli HA, Torres-Sánchez ED, Delgado-Lara DL, Pacheco-Moisés FP, Mireles-Ramírez MA, Torres-Mendoza BM, Moreno-Cih RI, Velázquez-Brizuela IE. Cognitive disorder and dementia in type 2 diabetes mellitus. World Journal of Diabetes. 2022;13(4):319.
43. Li S, Yang D, Zhou X, Chen L, Liu L, Lin R, Li X, Liu Y, Qiu H, Cao H, Liu J. Neurological and metabolic related pathophysiologies and treatment of comorbid diabetes with depression. CNS Neuroscience & Therapeutics. 2024;30(4):e14497.

44. Zhao X, Chen X, Yue C. Rutin ameliorates inflammation and oxidative stress in ulcerative colitis by inhibiting NLRP3 inflammasome signaling pathway. Cell Biochemistry and Biophysics. 2024;82(4):3715-26.
45. Qiu L, Gao C, Wang H, Ren Y, Li J, Li M, Du X, Li W, Zhang J. Effects of dietary polyphenol curcumin supplementation on metabolic, inflammatory, and oxidative stress indices in patients with metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. Frontiers in endocrinology. 2023;14:1216708.
46. Hu P, Li K, Peng XX, Kan Y, Yao TJ, Wang ZY, Li Z, Liu HY, Cai D. Curcumin derived from medicinal homologous foods: its main signals in immunoregulation of oxidative stress, inflammation, and apoptosis. Frontiers in immunology. 2023;14:1233652.
47. Imran M, Saeed F, Hussain G, Imran A, Mehmood Z, Gondal TA, El-Ghorab A, Ahmad I, Pezzani R, Arshad MU, Bacha U. Myricetin: A comprehensive review on its biological potentials. Food science & nutrition. 2021;9(10):5854-68.
48. Bássoli RM, Audi D, Ramalho BJ, Audi M, Quesada KR, Barbalho SM. The Effects of Curcumin on Neurodegenerative Diseases: A Systematic Review. Journal of Herbal Medicine. 2023;42:100771.
49. Islam MR, Rauf A, Akter S, Akter H, Al-Imran MI, Fakir MN, Thufa GK, Islam MT, Hemeg HA, Abdulmonem WA, Aljohani AS. Neuroprotective Potential of Curcumin in Neurodegenerative Diseases: Clinical Insights Into Cellular and Molecular Signaling Pathways. Journal of Biochemical and Molecular Toxicology. 2025;39(8):e70369.
50. Nicola MA, Attaai AH, Abdel-Raheem MH, Mohammed AF, Abu-Elhassan YF. Neuroprotective effects of rutin against cuprizone-induced multiple sclerosis in mice. Inflammopharmacology. 2024;32(2):1295-315.
51. Budzynska B, Faggio C, Kruk-Slomka M, Samec D, Nabavi SF, Sureda A, Devi KP, Nabavi SM. Rutin as neuroprotective agent: From bench to bedside. Current medicinal chemistry. 2019;26(27):5152-64.