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Myricetin Anti-diabetic Activity in 3T3 Cells Targeting the Nrf2-Keap Signaling Cascade

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Abstract

The study was aimed at assessing the effects of Myricetin, a potent anti-cancer compound, targets the NRF2-Keap1 pathway in 3T3-L1 fibroblast cells, which is crucial in cancer progression, cell growth, and metastasis. Various assays have demonstrated myricetin's therapeutic potential. Antioxidant properties, confirmed by the DPPH assay, show dose-dependent free radical inhibition, with myricetin achieving 69.98% inhibition at 500 µg/ml ($p<0.001$). Anti-inflammatory assays reveal significant reductions in inflammatory markers, with inhibition rising to 74.75% at the same concentration ($p<0.001$). Gene expression studies highlight myricetin's impact on key components of the NRF2/Keap1 pathway, essential for cancer cell survival. In 3T3-L1 cells treated with myricetin, notable changes were observed in mRNA expression levels: IR (0.95±0.05, $p<0.001$), IL-1β (0.96±0.04, $p<0.002$), Keap1 (0.9±0.04, $p<0.001$), Glut4 (0.6±0.04, $p<0.002$), NRF2 (0.96±0.4, $p<0.001$), and NFκB (0.94±0.05, $p<0.001$). These findings suggest myricetin disrupts critical pathways, contributing to reduced inflammation and potential cancer inhibition. The MTT assay further indicates no cytotoxicity after 48 hours, supporting its safety profile. Molecular docking studies reveal strong binding affinities of myricetin to key pathway components, with Keap1 showing the highest affinity (-9.7 kcal/mol), followed by IR (-8 kcal/mol), NFκB (-6.9 kcal/mol), and NRF2 (-6.9 kcal/mol). IL-1β and Glut4 showed affinities of -7.1 and -7.2 kcal/mol, respectively, reinforcing myricetin's role in modulating the NRF2/Keap1 pathway. In summary, myricetin's antioxidant, anti-inflammatory, and gene-modulatory activities, combined with its strong molecular interactions, position it as a promising therapeutic agent for both cancer and diabetes by modulating key cellular pathways.

Keywords: *Anti-diabetic, Anti-inflammatory, Antioxidant, Gene Expression, Health and Well-being, Myricetin, Novel Methods, NRF2-Keap Pathway.*

Introduction

Diabetes mellitus, a complex metabolic disorder, is characterized by persistent high blood glucose levels due to either insufficient insulin production (Type 1 diabetes) or the body's ineffective use of insulin (Type 2 diabetes). Type 2 diabetes, the more common form, often develops later in life and is closely linked to lifestyle factors such as obesity and physical inactivity [1]. Current treatment strategies aim to manage diabetes effectively through a combination of lifestyle modifications, medications, and monitoring. Lifestyle changes, including a balanced diet low in refined sugars and regular physical activity, play a crucial role in managing blood sugar levels. Medications for diabetes encompass a range of options, from oral

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medications like metformin and sulfonylureas to injectable insulin and newer classes such as GLP-1 receptor agonists and SGLT-2 inhibitors [2]. These drugs work by improving insulin sensitivity, enhancing insulin secretion, or reducing glucose absorption in the kidneys. Regular monitoring of blood glucose levels and HbA1c (average blood glucose over several months) helps individuals and healthcare providers adjust treatment plans to maintain optimal blood sugar control and prevent complications associated with diabetes, such as cardiovascular disease, nerve damage, and kidney problems. Advances in diabetes management continue to evolve, with ongoing research focusing on improving treatment efficacy, reducing side effects, and ultimately enhancing quality of life for those living with diabetes [3].

In the realm of diabetes therapy, advancements in molecular and pathway-targeted approaches have been pivotal in refining treatment strategies. One prominent molecular target in diabetes management is the insulin receptor and its downstream signalling pathways. Insulin resistance, a hallmark of Type 2 diabetes, occurs when cells fail to respond adequately to insulin, leading to impaired glucose uptake and elevated blood sugar levels [4]. Targeting the insulin receptor or its signalling intermediates aims to restore insulin sensitivity and improve glycemic control. Small molecule inhibitors that modulate insulin receptor signalling have shown promise in preclinical and clinical studies by enhancing cellular insulin response. Another significant pathway target in diabetes therapy is the incretin system, which regulates insulin secretion and glucose metabolism [5]. Incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are hormones released from the gut in response to food intake. They stimulate insulin release from pancreatic beta cells in a glucose-dependent manner, meaning they enhance insulin secretion only when blood glucose levels are elevated [6]. Targeted therapies involving GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors aim to enhance incretin signaling, thereby promoting glucose-dependent insulin secretion, inhibiting glucagon release, slowing gastric emptying, and promoting satiety. These therapies have been effective in lowering blood glucose levels, promoting weight loss, and reducing cardiovascular risk factors in individuals with Type 2 diabetes [7].

Myricetin, a flavonoid commonly found in fruits, vegetables, and medicinal herbs, plays a crucial biological role owing to its diverse pharmacological properties. As a potent antioxidant, myricetin scavenges free radicals and reduces oxidative stress, which is implicated in various chronic diseases including cancer, cardiovascular disorders, and neurodegenerative conditions [8, 9]. Its ability to inhibit inflammatory pathways and suppress pro-inflammatory cytokines contributes to its anti-inflammatory effects, potentially alleviating inflammation-related ailments such as arthritis and inflammatory bowel diseases. Moreover, myricetin has demonstrated antimicrobial activity against a range of pathogens, suggesting potential applications in combating microbial infections [10]. Beyond its antioxidative and anti-inflammatory actions, myricetin shows promise in modulating cellular signalling pathways involved in cancer progression, including inhibiting tumour cell proliferation and inducing apoptosis. Additionally, it has been studied for its neuroprotective effects, potentially mitigating neuronal damage and cognitive decline [11]. With its multifaceted pharmacological effects, myricetin continues to be an intriguing compound in biomedical research, offering therapeutic potential across various health conditions through its antioxidant, anti-inflammatory, antimicrobial, and potentially anti-cancer properties [12].

This study aims to comprehensively evaluate myricetin for its potential anti-inflammatory

and antioxidant properties. The research will involve a thorough screening process to assess these qualities using in vitro assays. Additionally, the study will investigate the impact of myricetin on cell growth and gene expression in 3T3-L1 cells. Preliminary findings indicate that myricetin does not significantly alter these cellular processes, highlighting its potential safety profile. Furthermore, molecular docking studies will be conducted to analyze the binding interactions between myricetin and the NRF2/Keap1 signalling pathway, crucial for regulating cellular responses to oxidative stress. These analyses aim to elucidate how myricetin modulates antioxidant defences within cells. The outcomes of this investigation hold promise for advancing myricetin as a therapeutic agent with dual anti-inflammatory and antioxidant properties. Such developments could lead to novel treatment options for managing inflammation-related disorders and addressing conditions linked to oxidative stress.

Materials and Methods

Chemical and Reagents

All chemicals and reagents employed in this study were obtained from reliable suppliers. Myricetin was purchased from Sigma-Aldrich Bangalore 560010, Karnataka, India. Gene-specific primers were procured from Eurofins Genomics Bangalore India. Cell line was procured from the National Centre for Cell Science (NCCS), Pune, India. DPPH was procured from SRL-chemicals, Chennai, India.

Dpph Radical Scavenging Activity

To assess myricetin's antioxidant properties, the DPPH radical scavenging assay was employed in this study [5]. The assay involved mixing 1.0 ml of a DPPH solution with 1.0 ml of the test extract across concentrations ranging from 0.1 to 0.5 mg/ml. Following incubation for a specified duration, the reaction mixture allowed the DPPH radicals to interact with the antioxidants present in the extract. The reduction in absorbance of the solution, indicative of scavenging activity, was measured at 517 nm using a spectrophotometer. Ascorbic acid, a well-established antioxidant, served as a standard at equivalent concentrations to those of the test extracts for comparative analysis.

DPPH (%) = (Control OD - Sample OD) × 100 / Control OD.

Anti-inflammatory Properties for Myricetin

The investigation into the anti-inflammatory potential involved assessing albumin denaturation inhibition, following a methodology adapted from Jayaraman et al. 2023 [5]. In this experimental process, a reaction mixture was prepared containing the test extracts and a 1% aqueous solution of bovine albumin fraction. To maintain the pH of the reaction mixture, a small amount of 1N HCl was added. The samples were then incubated at 37 °C for 20 minutes, followed by heating to 51°C for an additional 20 minutes. After cooling the samples to room temperature, turbidity was measured at 660 nm. This approach allowed for the evaluation of the extracts' ability to inhibit albumin denaturation, a key indicator of their potential anti-inflammatory activity.

Cell Culture

The 3T3-L1 cell line, sourced from the National Centre for Cell Science (NCCS), was cultured under standard conditions in a CO_2 incubator at 37 °C. The culture medium consisted of DMEM (Dulbecco’s Modified Eagle Medium) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin to support cell growth and viability. For the cytotoxicity assay, cells were detached using 0.25% trypsin-EDTA and subsequently seeded into 96-well plates.

Cytotoxicity by MTT Assay

This study investigated the cytotoxicity of myricetin, a natural compound, against the 3T3-L1 cell line using the MTT colourimetric assay.

Initially, cells were seeded at a density of $1x10^4$ cells per well in a 96-well plate and allowed to adhere overnight. Myricetin, which had been sterilized prior to use with high glucose (HG), was diluted in a 1% dimethyl sulfoxide (DMSO)-DMEM media mixture to achieve concentrations ranging from 10 to 100 µM. Subsequently, the diluted samples were added to the wells and incubated for 48 hours in a CO_2 incubator. After the incubation period, the supernatant was removed, and the wells were washed with 1X phosphate-buffered saline (PBS). MTT solution was then added to each well and incubated for 1 hour to allow formazan crystal formation. The formazan crystals were dissolved using DMSO, and the absorbance was measured at 590 nm to quantify cell viability. The percentage of viable cells was calculated relative to a control, and the data were analyzed and plotted using GraphPad Prism software. Statistical significance was evaluated using the paired Student's t-test to determine significant differences between experimental conditions.

Gene Expression Analysis

To quantify RNA content in the samples, spectrometric analysis was employed. For Real-Time PCR analysis, a meticulous reaction mixture was prepared using Takara SyBr green master mix. Specific forward and reverse primers, as detailed in Table 1, were carefully designed for the target genes. The experimental procedure included a melting analysis step, and a stable control was incorporated for normalization purposes. Results from this analysis were reported as fold changes relative to the control group. Real-time PCR was performed using the CFX96 Touch Real-Time PCR machine, ensuring precise quantification of gene expression levels. This robust methodology facilitated a thorough examination of the dynamics of gene expression under investigation, providing reliable insights into molecular changes within the samples.

Table 1. RT-PCR Primer used in this Study

Gene	Forward Primer (5' -> 3')	Reverse Primer (5' -> 3')	Reference
IR	AGGAGTGAGCTGAGCGAGAAG	CAGCCAGCTCAGAGAGCACA	[12]
IL-1β	GAGCACCTTCTTTCCCTTCATCTTT	TCATCTCGGAGCCTGTAGTGCAAGT	[13]
Keap-1	CAGACCTGGGAAATACCTGAGC	TGGAGTACGAGGCAGAAAGCC	[14]
Glut4	GCCCTGCCTTCTTCTTTCTG	GGGAGGGCAGAGTATGATGG	[15]
NRF2	CTGACGACCTAGGTTGCCAT	TCTTCTGCTTGGGTTTGGCA	[16]
Nfk-β	ATCGGAGAGCTTCTGCGTAG	TGAGGTAGTCTGGTAGCCGA	[17]
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG	[17]

Molecular Docking Analysis

For the docking analysis, crystal structures of several proteins were obtained from the Protein Data Bank (PDB), including IR (PDB ID: 6B57), IL-1β (PDB ID: 1JDH), Keap-1 (PDB ID: 2FLU), GLUT4, NRF2 (PDB ID: 6N6H), and Nfk-β (1VKX). These structures were accessed via https://www.pdb.org/pdb. During the docking simulations, a grid box measuring 90 Å × 90 Å × 90 Å with a spacing of 0.45 Å was employed. These specific parameters were selected to ensure precise calculations of drug molecule interactions with the target proteins. The 3D structural docking analyses were conducted using BIOVIA

Discovery Studio software, allowing for thorough visualization and analysis of the binding interactions between the compounds and the protein targets.

Pharmacokinetics Studies

In our research, we utilized in silico pharmacokinetic analysis to investigate the Absorption, Distribution, Metabolism, and Excretion (ADME) characteristics of myricetin. This analysis involved predicting several key parameters, such as molecular weight, topological polar surface area (TPSA), miLogP, the number of rotatable bonds, and the counts of hydrogen bond donors and acceptors, following Lipinski's rule of five (2001). The evaluation was conducted using a web-based tool available at www.swissadme.ch, specifically designed for calculating physicochemical descriptors and predicting pharmacokinetic properties of small molecules.

Statistical Analysis

The collected data underwent thorough analysis using the one-way analysis of variance (ANOVA) method. Post hoc comparisons were conducted using the Multiple Range tests by Duncan. A significance threshold of $P< 0.05$ was applied to determine statistical significance, ensuring robust interpretation of the experimental results. This approach facilitated detailed examination of group differences and identified significant findings with confidence.

Results

Effect of *Myricetin* on Anti-oxidant Activity

The investigation revealed a consistent trend in the inhibition of antioxidant activity, where inhibition rates increased proportionally with escalating concentrations of both myricetin and the standard compound, ranging from 100 to 500 µg/ml. The highest antioxidant effect for both the extract and the standard compound was observed at the maximum concentration of 500 µg/ml, as illustrated in Figure 1 and detailed in Table 2. Furthermore, the study's findings demonstrated a dose-dependent increase in the percentage of inhibition against DPPH radicals across concentrations from 100 to 500 µg/ml for both the extract and the standard substance. This pattern underscores the effectiveness of both myricetin and the standard compound in exhibiting antioxidant properties, particularly at higher concentrations, highlighting their potential therapeutic benefits in managing oxidative stress-related conditions.

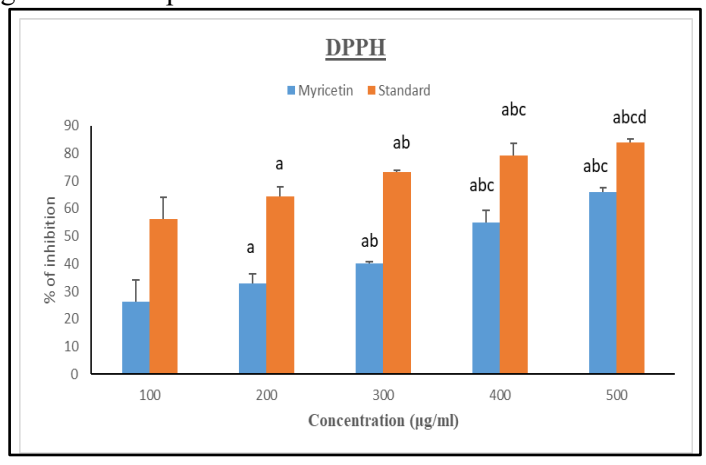

Figure 1. Represents the DPPH Activity (% of Inhibition)

Table 2. Represents the Dose-dependent Inhibition of DPPH by Myricetin

Sample concentration (µg/ml)	% of inhibition for myricetin	% of inhibition for standard
100	20.5±7.8	23±0.7

200	27.2±3.3[a]	33±1.2[a]
300	38.4±0.7[ab]	55±0.9[ab]
400	47.35±4.4[abc]	78±3.2[abc]
500	69.98±1.4[abc]	82.1±2.4[abcd]

Effect of *Myricetin* on Anti-inflammatory Activity

Similarly, the investigation revealed a consistent pattern in the inhibition of anti-inflammatory activity, where inhibition rates increased with higher concentrations (ranging from 100 to 500 µg/ml) of both the myricetin and the standard compound. The maximum anti-inflammatory efficacy for both the myricetin and the standard agent was observed at the concentration of 500 µg/ml, depicted in Figure 2 and detailed in Table 3. These findings highlight the dose-dependent nature of the anti-inflammatory effects exhibited by both myricetin and the standard compound, emphasizing their potential therapeutic significance in addressing inflammation-related conditions.

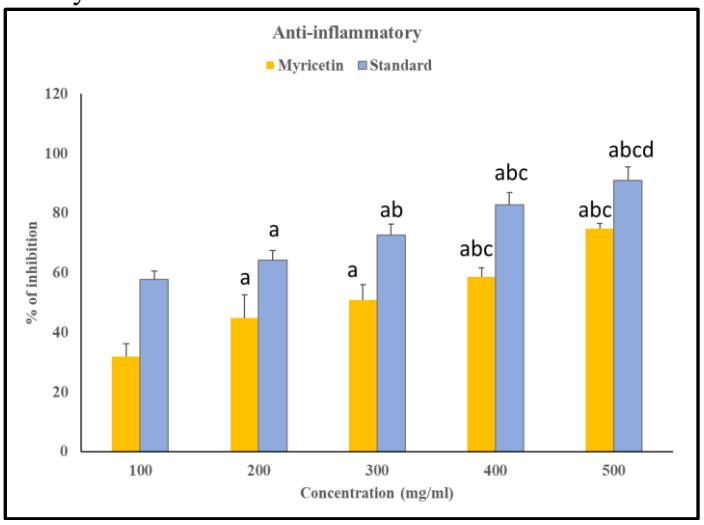

Figure 2. Represents the Anti-inflammatory Activity of Myricetin and Standard

Table 3. Represents the Anti-inflammatory Activity (% of Inhibition)

Sample concentration (µg/ml)	**% of inhibition for myricetin**	**% of inhibition for standard**
100	31.88±4.2	57.62±1.2
200	44.85±7.8 [a]	64.35±0.4[a]
300	50.87±5.2[ab]	72.58±4.8[ab]
400	58.6±3[abc]	82.73±1.33[abc]
500	74.75±1.8[abcd]	91.04±0.65[abcd]

Effect of *Myricetin* on Cytotoxicity

The cytotoxic impact of myricetin on 3T3-L1 cells, relevant to diabetic research, was assessed using the MTT assay. 3T3-L1 cells were exposed to increasing concentrations of myricetin, ranging from 0 to 100 µM for 48 h intervals. As illustrated in Figure 3, the results clearly indicate a significant and dose-dependent shows no cytotoxicity in cell

viability following exposure to myricetin over time (Figures 3 and 4). These findings underscore the time-dependent efficacy of myricetin in no toxicity and highlight its potential as a therapeutic agent in combating high glucose conditions.

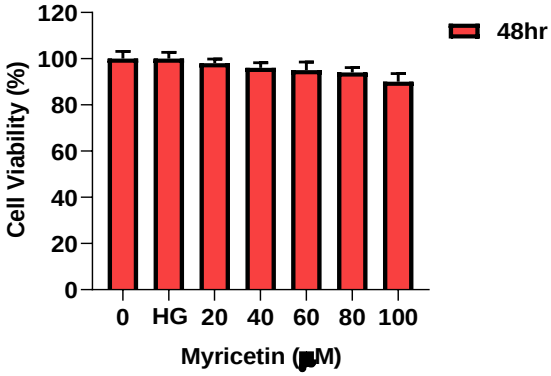

Figure 3. Cytotoxicity Assay of Cell Lines of 3T3-L1 Cells on 48 hrs

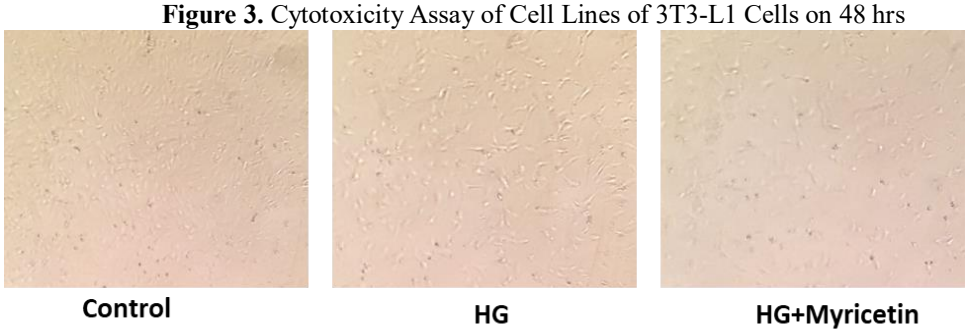

Figure 4. Morphological Changes of Myricetin Treated with 3T3-L1 Cells

Effect of *Myricetin* on Gene Expression

Having established that myricetin exhibits no cytotoxicity in 3T3-L1 cells, our study aimed to delve deeper into the molecular mechanisms influenced by myricetin in these cells. We specifically examined the molecular processes impacted by myricetin under conditions of high glucose, comparing scenarios both with and without myricetin treatment over 48 hours. Our investigation employed real-time PCR analysis, which revealed significant findings. In the presence of high glucose, myricetin treatment led to a notable decrease in the expression of several key genes, including insulin receptor (IR), interleukin-1β (IL-1β), Kelch-like ECH-associated protein 1 (Keap1), glucose transporter type 4 (Glut4), nuclear factor erythroid 2–related factor 2 (NRF2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) as shown in Figure 5. This downregulation suggests that myricetin may play a role in mitigating the detrimental effects associated with high glucose levels, which are often linked to inflammatory and oxidative stress responses. Interestingly, when comparing the high glucose condition with myricetin treatment to the high glucose condition alone, there was an elevated expression in the targeted genes. This indicates that myricetin may exert a complex regulatory effect on these molecular pathways, possibly involving feedback mechanisms or compensatory responses aimed at restoring cellular homeostasis. The collective results of our study suggest that myricetin treatment modulates the NRF2/Keap1 signalling pathway in 3T3-L1 cells. The NRF2/Keap1 pathway is a critical regulator of the antioxidant response, and its modulation by myricetin highlights its potential therapeutic benefits in managing oxidative stress and inflammation, particularly in the context of diabetes.

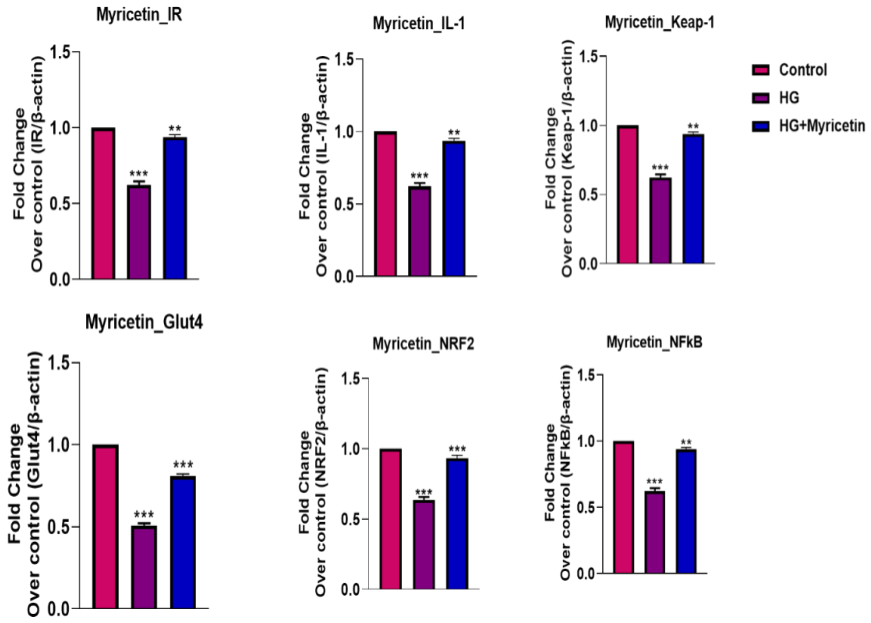

Figure 5. Effect of Myricetin on NFkB, IR, GLUT4, Nrf2, and Keap-1 mRNA Expression

Molecular Interaction of Myricetin with Insulin and Inflammatory Targets

The molecular docking simulations offered valuable insights into the interactions between myricetin and key proteins implicated in metastasis. The binding energies, as detailed in Table 4 and illustrated in Figure 6, demonstrate the strength and specificity of these interactions. myricetin exhibited notably high binding affinities with several critical proteins, including IR (-8 kcal/mol), NRF2 (-6.9 kcal/mol), Nfkβ (-6.9 kcal/mol), Keap1 (-9.7 kcal/mol), and IL-1β (-7.1 kcal/mol) (Table 4). These findings suggest that myricetin may significantly impact the Nrf2/Keap1 signalling pathway, which is crucial for regulating diabetes. The strong binding affinities observed indicate that myricetin forms stable complexes with these proteins, potentially disrupting the signalling mechanisms that promote diabetic management. The NRF2/Keap1 signalling pathway plays a pivotal role in various cellular processes, and its dysregulation is often associated with type 2 diabetic management.

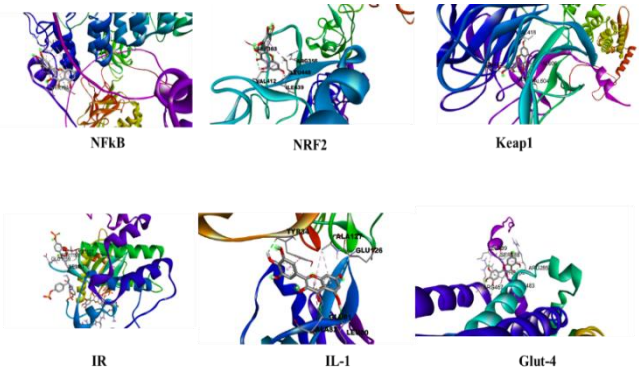

Figure 6. Molecular Docking Analysis of Selected Targets with Myricetin

Table 4. Binding Affinity Details of Selected Targets with Myricetin

Targets	Binding affinity (Kcal/mol)
NFkB	-6.9
NRF2	-6.9

IR	-8
Keap1	-9.7
IL-1	-7.1
Glut-4	-7.2

Myricetin Possess Biocompatibility in Pharmacokinetics Analysis

Lipinski's Rule of Five serves as a key guideline for evaluating the drug-likeness of chemical compounds, such as myricetin, particularly in assessing their potential as orally active drugs in humans. In our study, myricetin showed one violation related to molecular weight, hydrogen bond donors, and hydrogen bond acceptors among Lipinski's five criteria. These violations are detailed in Table 5. The Topological Polar Surface Area (TPSA) is essential for predicting the transport characteristics of drug candidates within the intestines and across the blood-brain barrier (BBB). With a TPSA value of 151.59, myricetin exhibited a notably high TPSA, suggesting a preference for hydrophilicity. These findings highlight potential challenges regarding myricetin's drug-likeness, emphasizing the need to consider factors such as TPSA and Lipinski's guidelines in the drug development process. Further optimization may be necessary to improve myricetin's pharmacokinetic properties and overall suitability as a therapeutic agent.

Table 5. ADME-T Properties of Myricetin

Compound	Myricetin	**ESOL Solubility (mol/l)**	9.88E-04
Synthetic Accessibility	3.27	**ESOL Class**	Soluble
Formula	C15H10O8	**Ali Log S**	-3.96
MW	318.24	**Ali Solubility (mg/ml)**	3.50E-02
#Heavy atoms	23	**Ali Solubility (mol/l)**	1.10E-04
#Aromatic heavy atoms	16	**Ali Class**	Soluble
Fraction Csp3	0	**Silicos-IT LogSw**	-2.66
#Rotatable bonds	1	**Silicos-IT Solubility (mg/ml)**	6.98E-01
#H-bond acceptors	8	**Silicos-IT Solubility (mol/l)**	2.19E-03
#H-bond donors	6	**Silicos-IT class**	Soluble
MR	80.06	**GI absorption**	Low
TPSA	151.59	**BBB permeant**	No
iLOGP	1.08	**Pgp substrate**	No
XLOGP3	1.18	**CYP1A2 inhibitor**	Yes
WLOGP	1.69	**CYP2C19 inhibitor**	No
MLOGP	-1.08	**CYP2C9 inhibitor**	No
Silicos-IT Log P	1.06	**CYP2D6 inhibitor**	No
Consensus Log P	0.79	**CYP3A4 inhibitor**	Yes
ESOL Log S	-3.01	**log Kp (cm/s)**	-7.4
ESOL Solubility (mg/ml)	3.14E-01	**Lipinski #violations**	1
Bioavailability Score	0.55	**Ghose #violations**	0
PAINS #alerts	1	**Veber #violations**	1
Brenk #alerts	1	**Egan #violations**	1
Leadlikeness #violations	0	**Muegge #violations**	2

Discussion

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, resulting from either an inadequate production of insulin (Type 1 diabetes) or an ineffective response to insulin (Type 2 diabetes) [2]. It poses a significant global health burden due to its associated complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy. The management of diabetes traditionally involves lifestyle modifications, oral hypoglycemic agents, and insulin therapy. However, the limitations of conventional treatments, such as side effects and patient non-compliance, have driven the exploration of alternative therapies. Phytotherapy, the use of plant-based compounds, has emerged as a promising adjunct or alternative in diabetes management [3, 6]. Various phytochemicals have demonstrated potential in regulating blood glucose levels, improving insulin sensitivity, and mitigating diabetes-related complications. For instance, compounds like berberine, curcumin, and myricetin exhibit antidiabetic properties by targeting multiple metabolic pathways, including enhancing insulin secretion, reducing insulin resistance, and exerting anti-inflammatory and antioxidant effects. These natural agents offer a multifaceted approach to diabetes management, potentially improving patient outcomes and quality of life. Extensive research into the efficacy, safety, and mechanisms of action of these phytochemicals continues to expand the therapeutic arsenal against diabetes, highlighting the need for integrating traditional knowledge with modern clinical practices [13-15]. The study found significantly higher expression levels of miR-7110 in oral squamous cell carcinoma (OSCC) tissues compared to adjacent normal tissues. The findings suggest that miR-7110 could be a promising early diagnostic or prognostic biomarker and a potential therapeutic target for OSCC [16].

The investigation revealed a consistent pattern in the inhibition of antioxidant and anti-inflammatory activities, with inhibition rates increasing proportionally with higher concentrations of both myricetin and a standard compound, ranging from 100 to 500 µg/ml. The highest antioxidant and anti-inflammatory effects for both the extract and the standard compound were observed at the maximum concentration of 500 µg/ml, as illustrated in Figures 1 and 2 and detailed in Tables 2 and 3, respectively. For antioxidant activity, a dose-dependent increase in the percentage of inhibition against DPPH radicals was noted, indicating that both myricetin and the standard compound effectively exhibit antioxidant properties, particularly at higher concentrations. Similarly, the anti-inflammatory activity showed a dose-dependent pattern, with increasing inhibition rates observed with higher concentrations of myricetin and the standard compound. These findings underscore the potential therapeutic benefits of myricetin in managing oxidative stress and inflammation-related conditions, highlighting its efficacy and dose-dependent nature in these roles.

By using SEM/EDX and FTIR analysis to verify the uniform distribution of nanoparticles, the study created an orthodontic glue that contained 1% green-synthesised titanium dioxide (TiO_2) nanoparticles. With 100% cell viability by day 14, the glue demonstrated decreased cytotoxicity against human gingival fibroblast cell lines, suggesting that it may improve antibacterial qualities and lessen white spot lesions in orthodontic treatment [17]. The cytotoxic impact of myricetin on 3T3-L1 cells, pertinent to diabetic research, was assessed using the MTT assay. Cells were exposed to increasing concentrations of myricetin, ranging from 0 to 100 µM, over 48-hour intervals. As shown in Figure 3, myricetin exhibited no cytotoxicity, maintaining cell viability over time. Interestingly, the cytotoxic effects of myricetin were less pronounced compared to

the high glucose and control groups after 48 hours of treatment, underscoring its safety and potential as a therapeutic agent under high glucose conditions. Building on these findings, our study delved into the molecular mechanisms influenced by myricetin in 3T3-L1 cells under high glucose conditions, with and without myricetin treatment over a 48-hour period. Real-time PCR analysis revealed that myricetin treatment in the presence of high glucose resulted in a notable decrease in the expression of several key genes, including insulin receptor (IR), interleukin-1β (IL-1β), Kelch-like ECH-associated protein 1 (Keap1), glucose transporter type 4 (Glut4), nuclear factor erythroid 2–related factor 2 (NRF2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), as illustrated in Figure 4. This downregulation suggests that myricetin may help mitigate the detrimental effects of high glucose levels, which are often linked to inflammatory and oxidative stress responses [18-22]. Sulfate-reducing bacteria (SRB) were found to be 20% more common in orthodontic patients than in healthy non-orthodontic volunteers. Of the 69 orthodontic patients, 14 had distinctive black precipitates that were suggestive of SRB. SRB colonization may be facilitated by stainless steel orthodontic appliances, which could have consequences for orthodontic metal corrosion [23]. Comparing high glucose conditions with and without myricetin treatment, there was an elevated expression of these genes in the myricetin-treated group, indicating a complex regulatory effect that may involve feedback mechanisms to restore cellular homeostasis. These results collectively suggest that myricetin modulates the NRF2/Keap1 signaling pathway in 3T3-L1 cells. The NRF2/Keap1 pathway is a critical regulator of the antioxidant response, and its modulation by myricetin highlights its potential therapeutic benefits in managing oxidative stress and inflammation, particularly in diabetes.

Conclusion

In conclusion, myricetin is a potent and safe therapeutic compound that effectively targets the NRF2-Keap1 pathway in 3T3-L1 normal fibroblast cells, crucial for insulin signaling. Assays confirm myricetin's strong antioxidant properties, significant anti-inflammatory effects, and modulation of key genes in the NRF2/Keap1 pathway, such as IR, IL-1β, Keap1, Glut4, NRF2, and NF-κβ. The DPPH assay highlights its ability to neutralize free radicals, while anti-inflammatory assays show reduced inflammatory markers. The MTT assay indicates no adverse effects on cell viability after 48 hours, confirming its safety. Docking studies reveal strong binding affinity with NRF2/Keap1 components. Overall, myricetin's antioxidant, anti-inflammatory, and gene modulation effects highlight its potential in managing diabetes and inflammation-related conditions.

Conflict of Interest

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

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