

Rutin modulates the TGR5/GLP1 pathway and downregulates proinflammatory cytokines genes in streptozotocin-induced diabetic rats

Olusola Olalekan Elekofehinti^a, Olorunfemi Raphael Molehin^b, Moses Orimoloye Akinjiyan^{a*} and Aderonke Elizabeth Fakayode^a

^aBioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria

^bDepartment of Biochemistry, Ekiti State University, PMB 5363, Ado-Ekiti, Ekiti State, Nigeria

*Corresponding author: Moses Orimoloye Akinjiyan, Bioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria. E-mail: moakinjiyan@futa.edu.ng

DOI: 10.26599/JFB.2024.95027390

Received: June 26, 2024; Revised received & accepted: September 18, 2024

Abbreviations: STZ, streptozotocin; PDX-1, Pancreatic and duodenal home box 1; TGR-5, Takeda G protein-coupled receptor 5; GLP-1, glucagon-like peptide-1; DPP4, dipeptidyl peptidase-4; IL-1 β and IL-6, interleukin 1 beta and interleukin 6 respectively; TNF- α , tumour necrosis factor-alpha; mRNA, messenger ribonucleic acid; RT-PCR, Reverse transcriptase-polymerase chain reaction

Citation: Elekofehinti, O.O., Molehin, O.R., Akinjiyan, M.O., and Fakayode, A.E. (2024). Rutin modulates the TGR5/GLP1 pathway and downregulates proinflammatory cytokines genes in streptozotocin-induced diabetic rats. *J. Food Bioact.* 27: 88–103.

Abstract

Rutin, a bioactive component of citrus has been used to treat diabetes mellitus, but there is a paucity of information on the mechanism. In this study, rutin's impact on the expression of genes related to inflammation and insulin secretion were examined. Rats induced with streptozotocin (60 mg/kg) were grouped into six: normal control, diabetes untreated, metformin and rutin-treated groups (25, 50, and 100 mg/kg), for 28 days. The blood glucose were evaluated at 3-day intervals with glucometer. The mRNA expression of PDX-1, TGR-5, GLP-1, DPP4, TNF- α , IL-1 β and IL-6 was investigated utilizing RT-PCR. Schrödinger suites was used to dock rutin with TGR-5, GLP-1 and DPP4. After treatment, the groups administered with rutin had decreased blood glucose levels than the diabetes untreated group. Compared with those in the untreated diabetes group, the expression of PDX-1, TGR-5 (25 and 100 mg/kg), and GLP-1 (25 and 100 mg/kg) was significantly upregulated, and the expressions of DPP4, IL-1 β , TNF- α , and IL-6 were significantly downregulated in the 25 mg/kg rutin-treated group. The docking results showed that rutin is a potent ligand of the selected proteins investigated. The antidiabetic effects of rutin could be linked to its potential to downregulate proinflammatory cytokines and modulate the TGR5/GLP-1 signally, leading to normoglycemia.

Keywords: Rutin; diabetes mellitus; gene expression; inflammation; molecular docking.

1. Introduction

The most common sign of diabetes mellitus (DM), a collection of metabolic diseases, is persistent hyperglycemia (high blood glucose), which can be caused by insufficient insulin production or improper cell response to insulin (Lim et al., 2021; Song et al., 2022; Ojo et al., 2023). Glucose, though present in the blood in

diabetic patients, is unable to be fully incorporated and utilized by mitochondria, the major site of energy and ATP generation. DM is a significant global health issue and one of the top six causes of death globally, accounting for around 2 million deaths yearly (Talib et al., 2021). According to estimates, there were 422 million adults worldwide with diabetes in 2014, and 463 million in 2019. This figure is expected to be 593 million by 2035 and 643 million

by 2040 (Zimmet et al., 2016)

Due to their high therapeutic potential and the presence of phytochemicals like flavonoids, medicinal and traditional plants have been employed for a long time to manage a variety of conditions, including diabetes mellitus (Ahn, 2017; Ijatuyi et al., 2024). Flavonoids are naturally occurring plant-based products with a wide range of biological and therapeutic uses. This broad class of substances exhibits hepatoprotective, nephroprotective, anticarcinogenic, antihyperglycemic, and antihyperlipidemic properties (Mutha et al., 2021). These are a class of naturally occurring substances with polyphenolic structures that are commonly present in fruits and vegetables and are also added to some drinks as naturally derived colours. The fundamental structure of flavonoids includes fifteen carbon atoms and two phenolic rings carrying one or more hydroxyl groups (Mahmoud et al., 2019). Flavonoids have two aromatic rings attached to a heterocyclic pyrane ring (Mahmoud et al., 2019). These compounds are rich in double bonds and include rutin, hesperidin, hesperetin, and eriodictyol among others. Recently, studies have been carried out on these phytochemicals to determine their mode and mechanism of action. Rutin is a citrus flavonoid that has many therapeutic effects and has been used to manage DM (Mahmoud et al., 2019; Liang et al., 2021).

Mitochondria are the site of cellular respiration and this organelle is also often referred to as the powerhouse of a cell because the energy it generates powers other cell activities (Kim et al., 2008; Anderson et al., 2019). This energy is generated by the breakdown of glucose, a process known as glycolysis (Navale and Paranjape, 2016). Because of its continuous activity, it is the site of free radical and pro-oxidant generation, which is further increased by hyperglycemic conditions in DM. This results in oxidative stress and inflammation.

Pancreatic and duodenal home box 1 (PDX-1) is a gene associated with mitochondrial regeneration in the pancreas (Wang and Farhana, 2023). The antidiabetic mechanism of rutin action was investigated in this study on genes associated with mitochondrial biogenesis, insulin secretion, and inflammation in the pancreas and liver of STZ-induced rats. The pancreas is a key organ involved in glucose regulation since its beta-cells secrete insulin via Takeda G-protein-coupled receptor 5 (TGR 5) and glucagon-like peptide 1 (GLP-1) signally, insulin hormone is crucial in glucose metabolism (Edgerton et al., 2021). The liver helps in the distribution of glucose throughout the body, and it also stores excess glucose in the form of glycogen (Nakrani et al., 2023). The genes associated with insulin sensitivity and glucose metabolism examined in this study included TGR 5, GLP-1, and dipeptidyl peptidase-4 (DPP4) (Cai, et al., 2021). Genes linked to inflammation in pancreatic and hepatic cells, such as interleukin-6 (IL-6), interleukin-1beta (IL-1 β), and tumour necrosis factor-alpha (TNF- α), were also investigated in this study.

2. Materials and methods

2.1. Materials

Analytical grade solvents and reagents were employed in the investigation. The sources of streptozotocin and rutin were Sigma-Aldrich (St. Louis, MO, USA). From Inqaba Biotech, Africa Genomics Company (South Africa), we acquired TRIzol, reverse transcriptase, specific gene primers, loading buffer, DNA ladder, nuclease-free water, agarose gel powder, EZ Vision, ethidium bromide, and gel loading dye. We bought regular rat feed and metformin from local suppliers.

2.2. Animals

Male Wistar rats used in the experiment were obtained from the University of Ibadan in Nigeria, weighing an average of 189.33 ± 5 g. The rats were acclimated for two weeks at room temperature in galvanized cages at the animal house of the Department of Biochemistry, Federal University of Technology, Akure. The rats were kept on a 12-hour light/12-hour dark cycle, with free access to water and standard rat chow (Oloyede et al., 2022). The National Institutes of Health's (NIH) Guidelines for the Care and Use of Laboratory Animals were adhered to when working with the animals.

2.3. Experimental design

The experimental rats were then intraperitoneally induced with streptozotocin (60 mg/kg) in 0.1 molar citrate buffer (pH 4.5) with the aid of a needle and syringe (Ghasemi and Jeddi, 2023). Streptozotocin (60 mg/kg) is widely reported to reduce beta-cell levels, and this destructive ability is believed to be caused by a continuous cycle of oxidative stress (Nahdi et al., 2017). The rats' blood sugar was devaluated with a glucometer (ACCU-CHEK) and glucose strip after 72 hrs (3 days) of induction, with readings of rats equal to or greater than 200 mg/dl considered diabetic. They were then put into six groups viz:

- Group 1 = the normal control group;
- Group 2 = diabetic control group;
- Group 3 = 100 mg/kg metformin (standard drug);
- Group 4 = 25 mg/kg rutin treatment;
- Group 5 = 50 mg/kg rutin treatment;
- Group 6 = 100 mg/kg rutin treatment.

The rats were treated daily orally with the aid of gavage as described above for 28 days (31 days after induction), and the rats' blood sugar and body weight mass were evaluated at 3-day intervals. After that, the mice were sacrificed, and the tissues of interest were harvested as described previously by (Elekofehinti and Akinjiyan, 2020).

2.4. Gene expression

Using TRIzol reagent (Thermo Fisher Scientific), RNA was extracted from the tissues and then converted to cDNA using a Proto Script First Strand cDNA Synthesis Kit (NEB). Using the primer sets listed in Table 1, OneTaq[®]2X Master Mix (NEB) was used to create PCR amplicons. The amplification of selected genes of interest was performed using a thermocycler, followed by agarose gel electrophoresis of the sample amplicons.

The pancreas was examined for the expression of the genes for dipeptidyl peptidase-4 (DPP4), glucagon-like peptide-1 receptor (GLP-1), Takeda-G-protein receptor-5 (TGR-5), pancreatic and duodenal home box 1 (PDX-1), and interleukin 6 (IL-6). Both the pancreas and the liver were used to study the expression of interleukin-1 beta (IL-1 β) and tumour necrosis factor α (TNF- α).

2.5. Agarose gel electrophoresis

One per cent agarose gel electrophoresis was applied to polymerase chain reaction amplicons. A camera was used to take pictures of the gels under an ultraviolet (UV) transilluminator, revealing their band intensities.

Table 1. Gene expression primer sets

Gene	Forward primers	Reverse primers
PDX-1	5'-CCTTCCCGAATGGAACCGA-3'	5'-GTATGCACCTCTGCCAC-3'
TGR-5	5'-TGTCACACAACACCACTGAG-3'	5'-CAAGCAGGGAGAGGAAACAA-3'
GLP-1	5'-TCCCAAAGGAGCTCCACCTG-3'	5'-TTCTCTCCGTGTCTTGAGGG-3'
DPP-4	5'-GCAAGACGTGGGTAATGATG-3'	5'-AGCCTGGTTGGGTTTGTATG-3'
TNF α	5'-ACCACGCTCTTCTGTCTACTG-3'	5'-CTTGGTGGTTTGTCTACGAC-3'
IL-6	5'-TCTCTCCGCAAGAGACTTCCA-3'	5'-ATACTGGTCTGTTGTGGGTGG-3'
IL-1 β	5'-CCGGATGGGTAGGATAAAGTT-3'	5'-ACCACTGAGGTAGGAAAGA-3'
GAPDH	5'-AGACAGCCGCATCTTCTTGT-3'	5'-CTTGCCGTGGGTAGATCAT-3'

2.6. Molecular docking

2.6.1. Ligand Preparation

The rutin compound's structure was obtained from <https://pubchem.ncbi.nlm.nih.gov/> and ready for docking in Maestro, and Schrödinger suites using the LigPrep module. Appropriately chiral low-energy three-dimensional structures were produced. At a physiological pH of 7.2 ± 0.2 , the likely ionization energy for the rutin ligand structure was generated and reduced with the OPLS3 force field (Iwaloye et al., 2021).

2.6.2. Molecular docking study

Using the "receptor grid generation" option in the glide-v7.5 program of Maestro-v11.5, receptor grids were created around the binding sites of Takeda-G-protein-receptor-5 (PDB ID: 7BWO), glucagon-like peptide-1 receptor (PDB ID: 6XLA), and dipeptidyl peptidase-4 (DPP4) (PDB ID: 2OQV). Next, using the Schrödinger suite workflow module's extra precision (XP) workflow module with default parameters, the prepared rutin ligands were docked into the receptor grids. Maestro's virtual screening workflow was uti-

lized to score and dock rutin with specific receptors linked to insulin regulation (Arowosegbe et al., 2020; Elekofehinti et al., 2024).

2.7. Data analysis

Densitometric quantification of the band intensities were performed using ImageJ software (<http://imagej.en.softonic.com/>). The mean \pm standard error of the mean (SEM) was used to represent the results. PRISM 5.01 (GraphPad Software, Inc.) was utilized for these statistical analyses, which involved one-way analysis of variance (ANOVA) and post-hoc Dunnett's multiple range test, with a statistically significant variance at $p < 0.05$.

3. Results

3.1. Effect of rutin administration on the fasting blood glucose of streptozotocin-induced diabetic rats

The effects of rutin on blood glucose levels in type 1 diabetic rats are displayed in Figure 1. Three days after induction, blood glucose levels in the induced group were significantly higher than in the non-

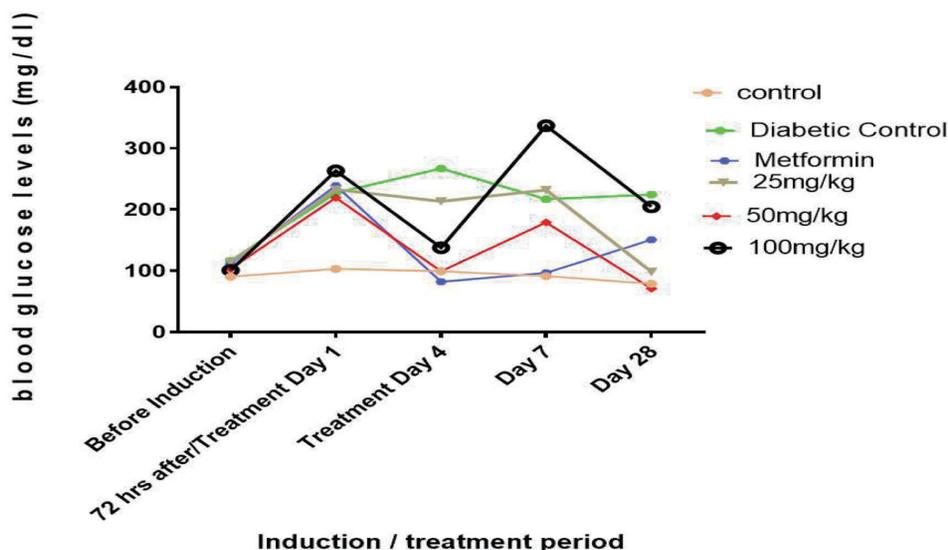


Figure 1. Line graphs showing the hypoglycemic effect of rutin on blood glucose levels in STZ-induced diabetic rats.

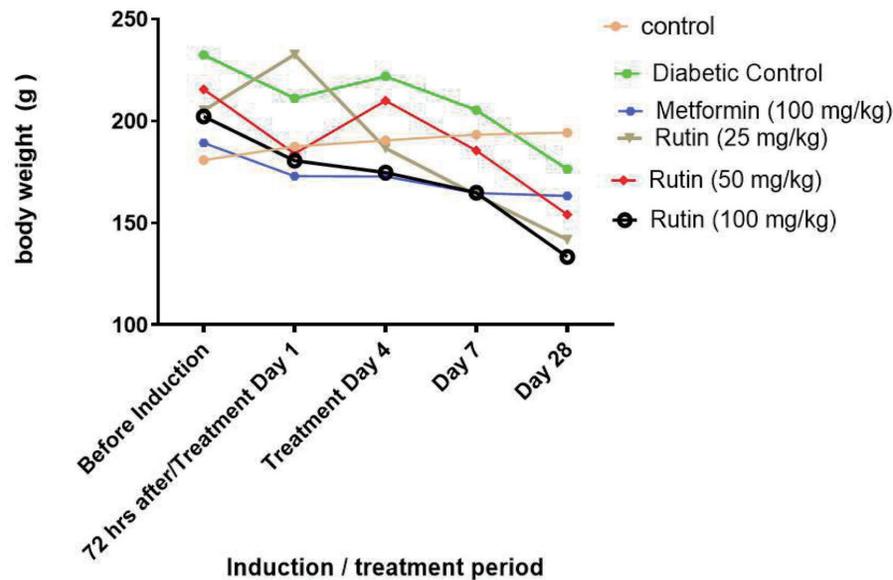


Figure 2. Line charts showing the effect of rutin on the weight of rats with streptozotocin-induced diabetes.

diabetic control group due to diabetes induced by STZ (Figure 1, $p < 0.05$). When rutin (25 and 50 mg/kg) and metformin were administered for 28 days (31 days after induction), the blood glucose concentrations of the treated diabetic groups were significantly lower than those of the group receiving no treatment for diabetes.

3.2. Rutin treatment and weight patterns of streptozotocin-induced diabetic rats

After 28 days of treatment, diabetic control rats lost weight in comparison to non-diabetic control rats (Figure 2). After 28 days of treatment, the rutin-treated group lost weight in comparison to the non-diabetic control group.

3.3. Effect of rutin on genes associated with mitochondrial biogenesis in the pancreas of STZ-induced diabetic rats

Pancreatic and duodenal home box 1 (PDX-1) was significantly upregulated (Figure 3, $p < 0.05$) in the Rutin-treated group compared with the STZ-induced diabetic nontreated and metformin-treated groups at all tested concentrations. This finding suggested the regenerative ability of rutin on pancreatic mitochondria and function.

3.4. Effect of the oral administration of rutin on genes linked to inflammation in the pancreas of streptozotocin-induced diabetic rats

Tumor necrosis factor alpha (TNF- α) and interleukin-1 beta (IL-1 β) are proinflammatory cytokines. The expression of these genes in the pancreas was investigated in the present study. The expression of these genes was significantly ($p < 0.05$) greater in diabetic control rats than in control rats (Figures 4 and 5), which indicates toxicity. Treatment with 25 or 100 mg/kg rutin significantly downregulated IL-1 β compared to the diabetic control group. Metformin and rutin (25 and 50 mg/kg) significantly repressed TNF- α relative to the diabetic control class after 28 days of rutin administration.

3.5. Effect of oral administration of rutin on genes associated with inflammation in the liver of rats with streptozotocin (STZ)-induced diabetes

IL-1 β , interleukin-6 (IL-6) and TNF- α expressions were significantly upregulated upon STZ induction compared to that in the noninduced control group. Rutin at 25 mg/kg notably downregulated the expression of these three cytokines in the liver (Figures 6–8). This suggests rutin's potential to reduce inflammation brought on by diabetes.

3.6. Effect of rutin on insulin-sensitive genes in the pancreas of streptozotocin-induced diabetic rats

TGR-5, GLP-1 and DPP4 genes were investigated in the pancreas (Figures 9–11). These genes are associated with glucose metabolism. After 28 days of treatment, rutin (50 and 100 mg/kg) significantly upregulated TGR-5 expression relative to that in the diabetic control group. Metformin and rutin (25 and 50 mg/kg) significantly upregulated GLP-1 ($p < 0.05$) compared to that in the STZ-induced diabetic untreated group. When compared to the nondiabetic control group, the expression of dipeptidyl peptidase-4 (DPP4) was significantly upregulated in the diabetic control and metformin-treated groups, suggesting a disruption in glucose metabolism. However, rutin (25, 50, and 100 mg/kg) significantly corrected this abnormality, as shown by the downregulation of DPP4 expression in comparison to the diabetic control group (Figure 11, $p < 0.05$).

3.7. In Silico molecular interactions of rutin with selected proteins

A molecular docking simulation of rutin was performed with receptor targets associated with insulin sensitivity, such as GLP-1, TGR-5 and DPP4 (Figures 12–17). The results revealed that rutin is a potent ligand of these targets (Table 2). The binding affinities of TGR-5 and GLP-1 were -12.407 kcal/mol and -12.723 kcal/mol,

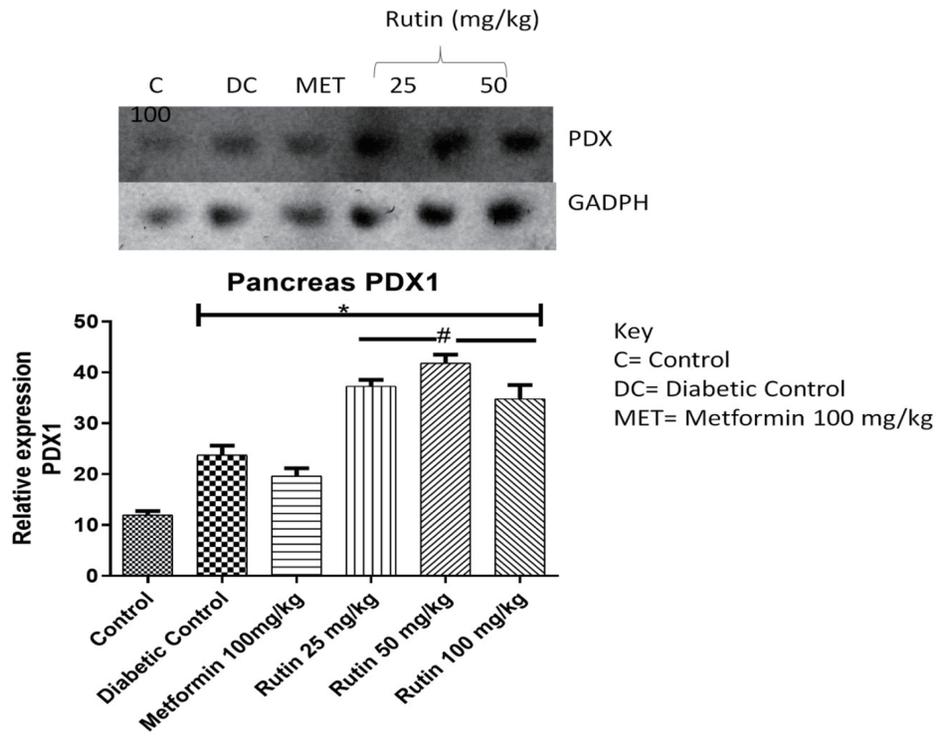


Figure 3. Pictorial view of RT-PCR agarose gel electrophoresis (AGE) for pancreatic and duodenal home box 1 (PDX-1) in rats with STZ-induced diabetes treated with rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

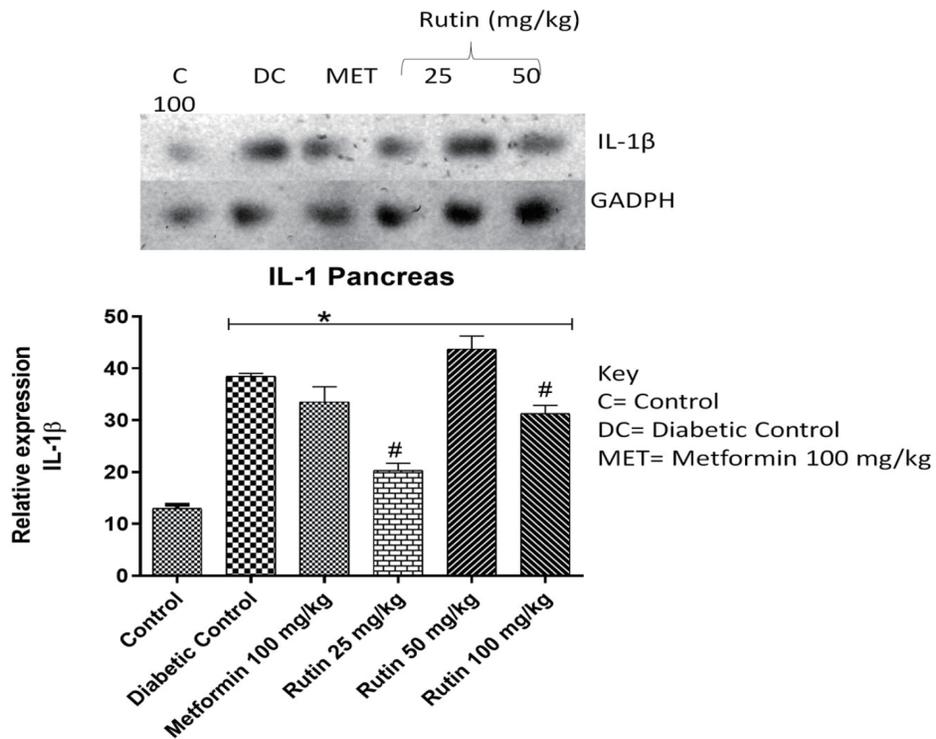


Figure 4. Pictorial view of RT-PCR AGE analysis of pancreatic interleukin-1 beta expression in rats with STZ-induced diabetes treated with rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

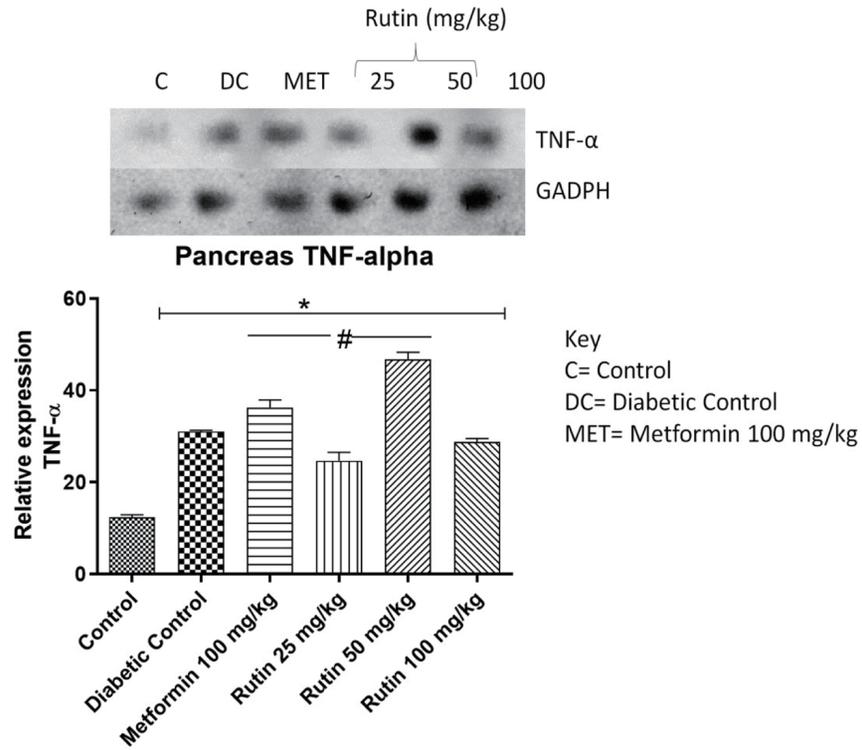


Figure 5. Pictorial view of RT-PCR AGE analysis of pancreatic TNF-α expression in STZ-induced diabetic rats orally administered rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

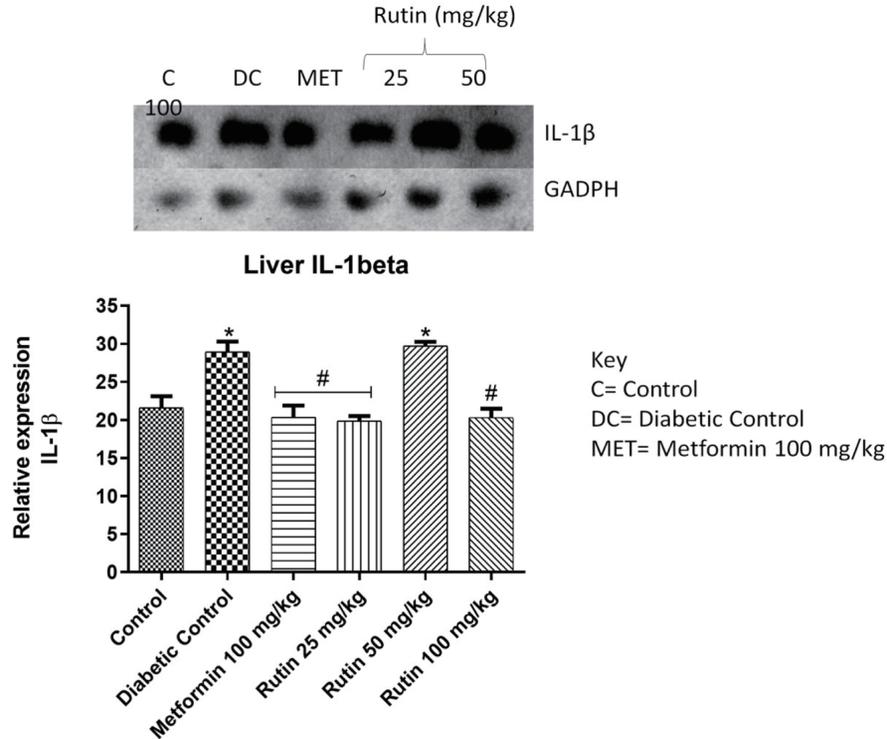


Figure 6. Pictorial view of RT-PCR AGE analysis of hepatic interleukin-1 beta expression in rats with STZ-induced diabetes treated with rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

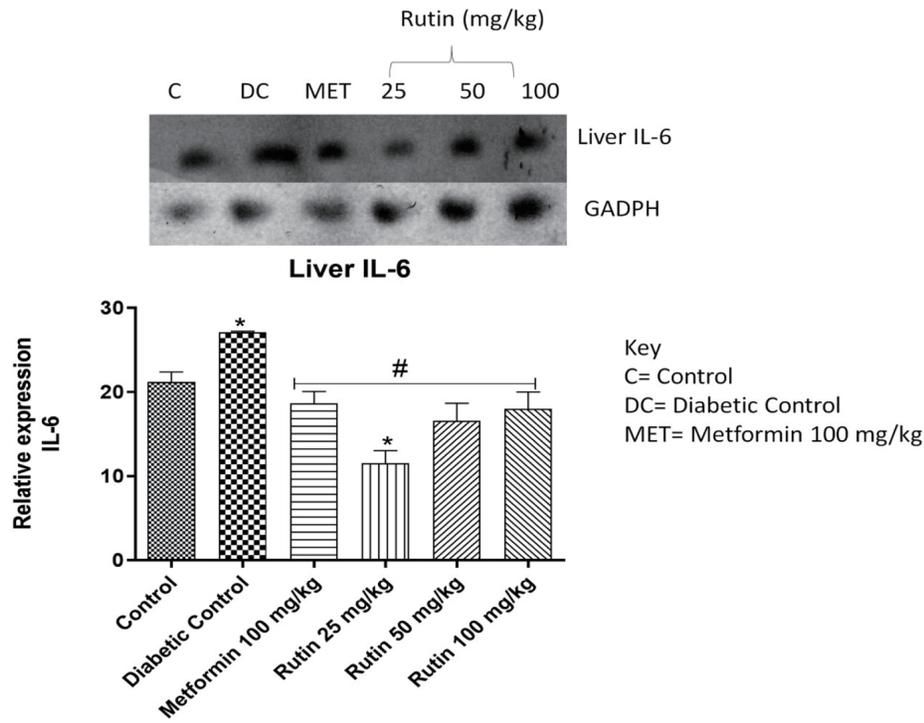


Figure 7. Pictorial view of RT-PCR AGE for interleukin-6 expression in rats with STZ-induced diabetes (n = 3) treated with rutin followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

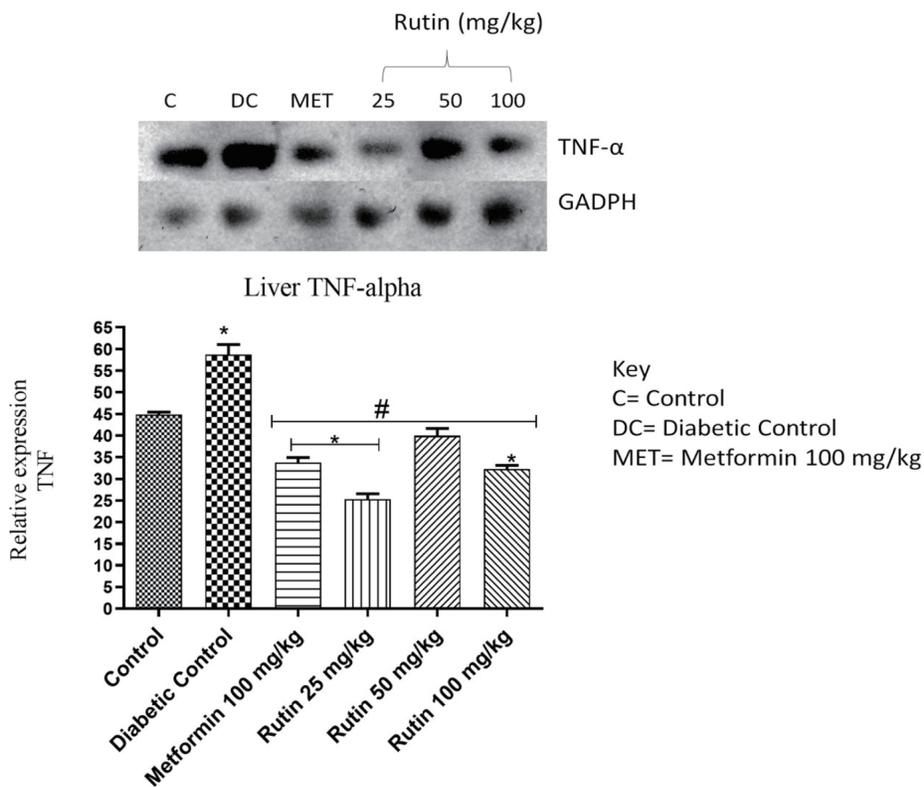


Figure 8. Pictorial view of RT-PCR AGE for hepatic tumour necrosis factor (TNF- α) in rats with STZ-induced diabetes treated with rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

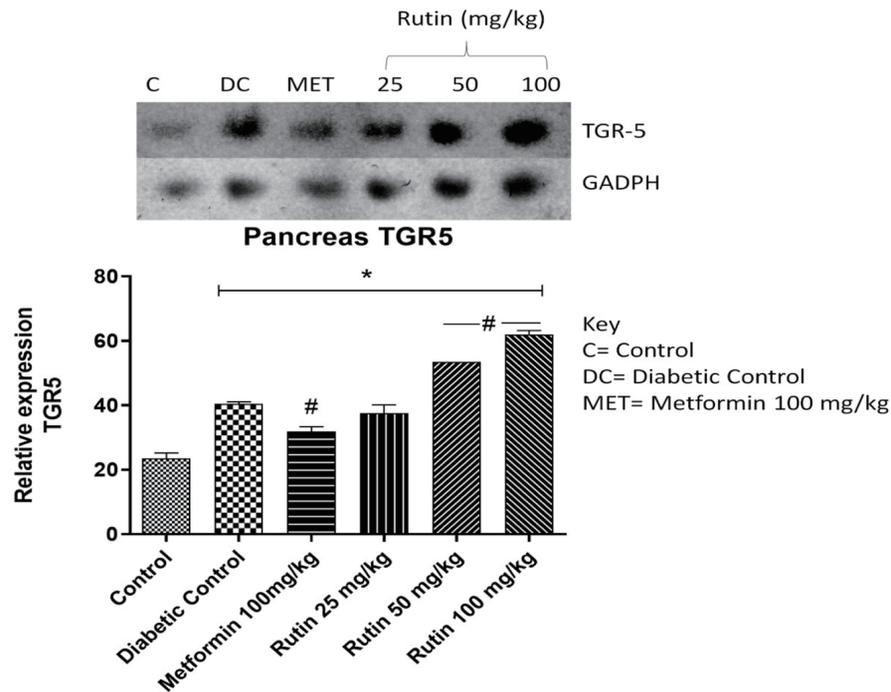


Figure 9. Pictorial view of RT-PCR AGE for pancreatic Takeda G-protein-coupled receptor 5 (TGR5) in rats with STZ-induced diabetes treated with rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

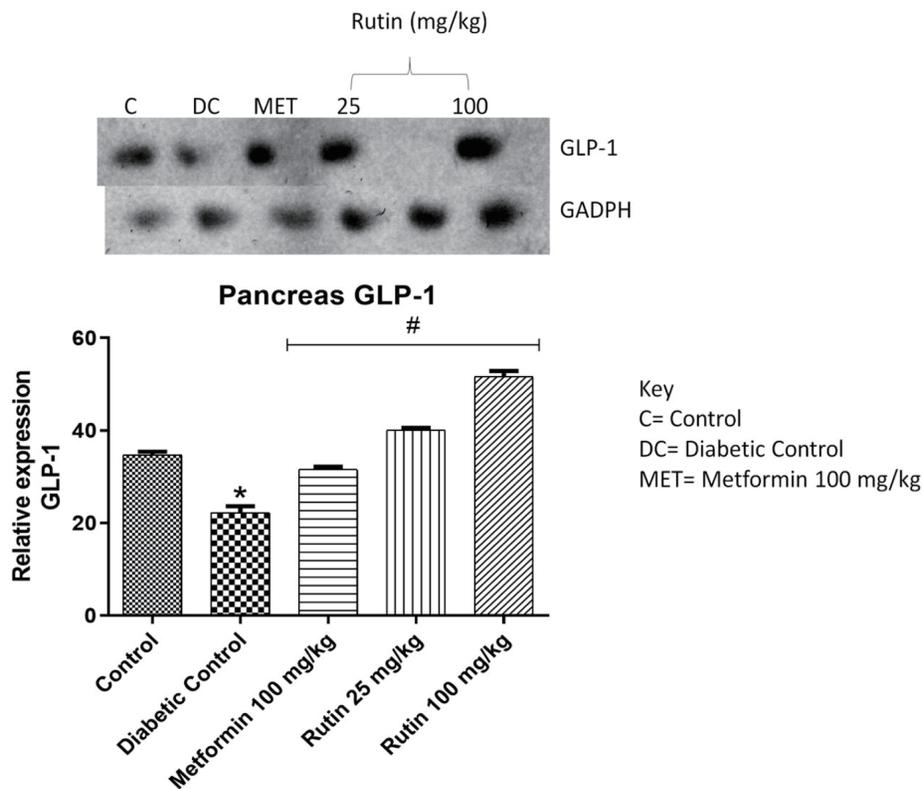


Figure 10. Pictorial view of RT-PCR AGE for pancreatic glucagon-like peptide-1 (GLP-1) in rats with STZ-induced diabetes treated with rutin (n = 3) followed by densitometric analysis. * and # represent a significant variation between the control group and the diabetic control group respectively at $p < 0.05$.

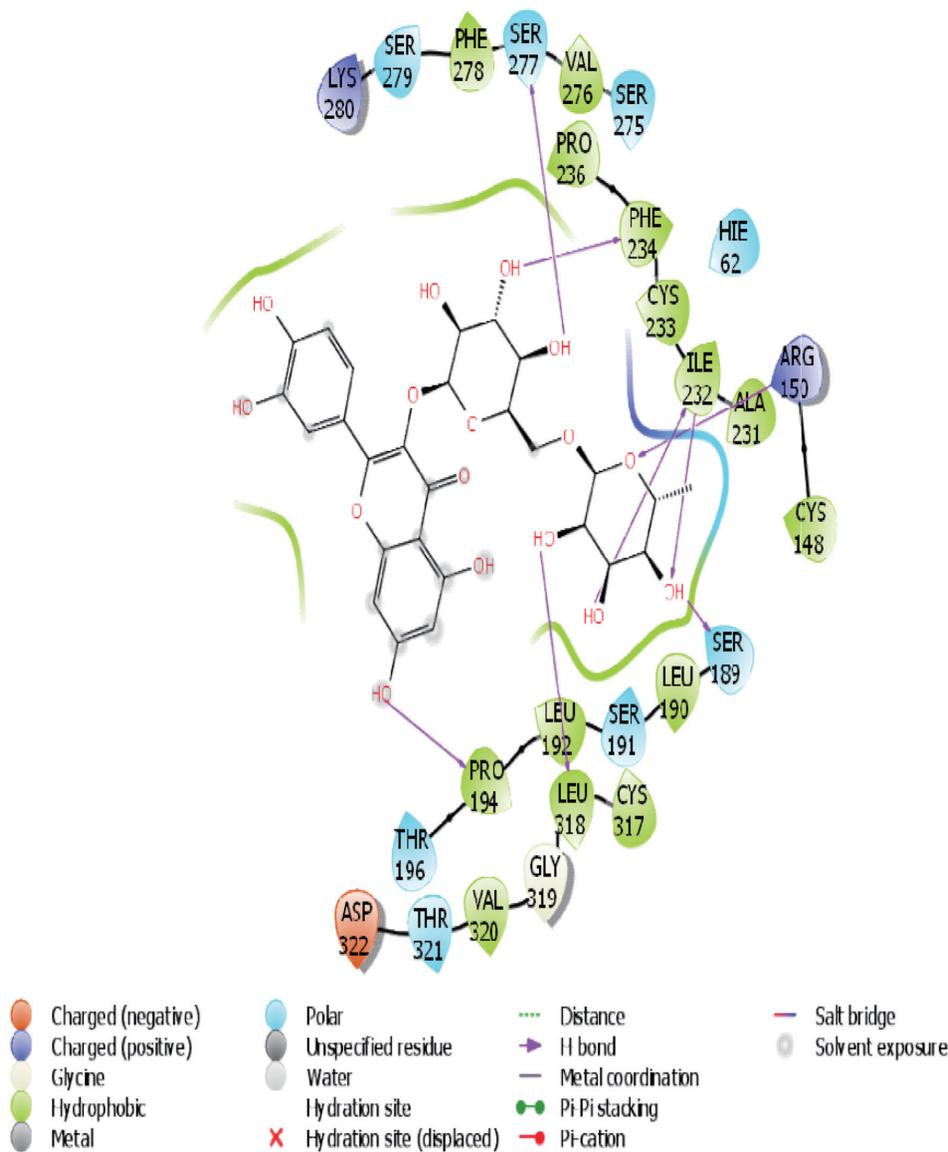


Figure 13. The 2D chemical relationship of Rutin with TGR5 with amino acid residues.

respectively, when rutin was docked to their 3D structures. The phytochemicals' binding position and binding site with the insulin receptor are depicted in Panel A (3D image) of the following Figures 12 to 17, while Panel B (2D image) depicts the ligand rutin's molecular interaction with amino acid residues inside the protein structures' binding pocket.

Table 2 shows the binding affinities of rutin with the listed protein and receptor associated with insulin sensitivity. This figure also shows the amino acids involved in the hydrophobic interactions. A number of hydrogen bonds and Pi-Pi.

4. Discussion

Mitochondria are highly dependent on glucose as a substrate for their energy-generating activities (Anderson et al., 2019). The electron transport chain and oxidative phosphorylation are two

key reactions that occur in mitochondria. Nicotinamide adenine dinucleotide (NADH) is needed for the electron transport chain and oxidative phosphorylation, which occurs in the mitochondria for adenosine triphosphate (ATP) or energy generation (Elekofehinti et al., 2024). NADH is generated in the cytosol during glycolysis. However, NADH production is reduced or made impossible in diabetic patients because of glucose intolerance (Deshpande and Mohiuddin, 2021; Wu et al., 2017). This eventually leads to mitochondrial degeneration and shutdown. Eventually, cell death can occur, especially in the beta-cells of the pancreas, since the powerhouse of the cell has been shut down. The co-enzymes and co-factors above are found and abundant in the glycolytic pathway which can be initiated by the hormone insulin. Insulin secretion in turn can be initiated by the TGR5 which then signals the GLP-1.

The blood sugar levels of the experimental mammals were significantly ($p < 0.05$) elevated three days after STZ induction, as shown in Figure 1. This could be due to streptozotocin (STZ),

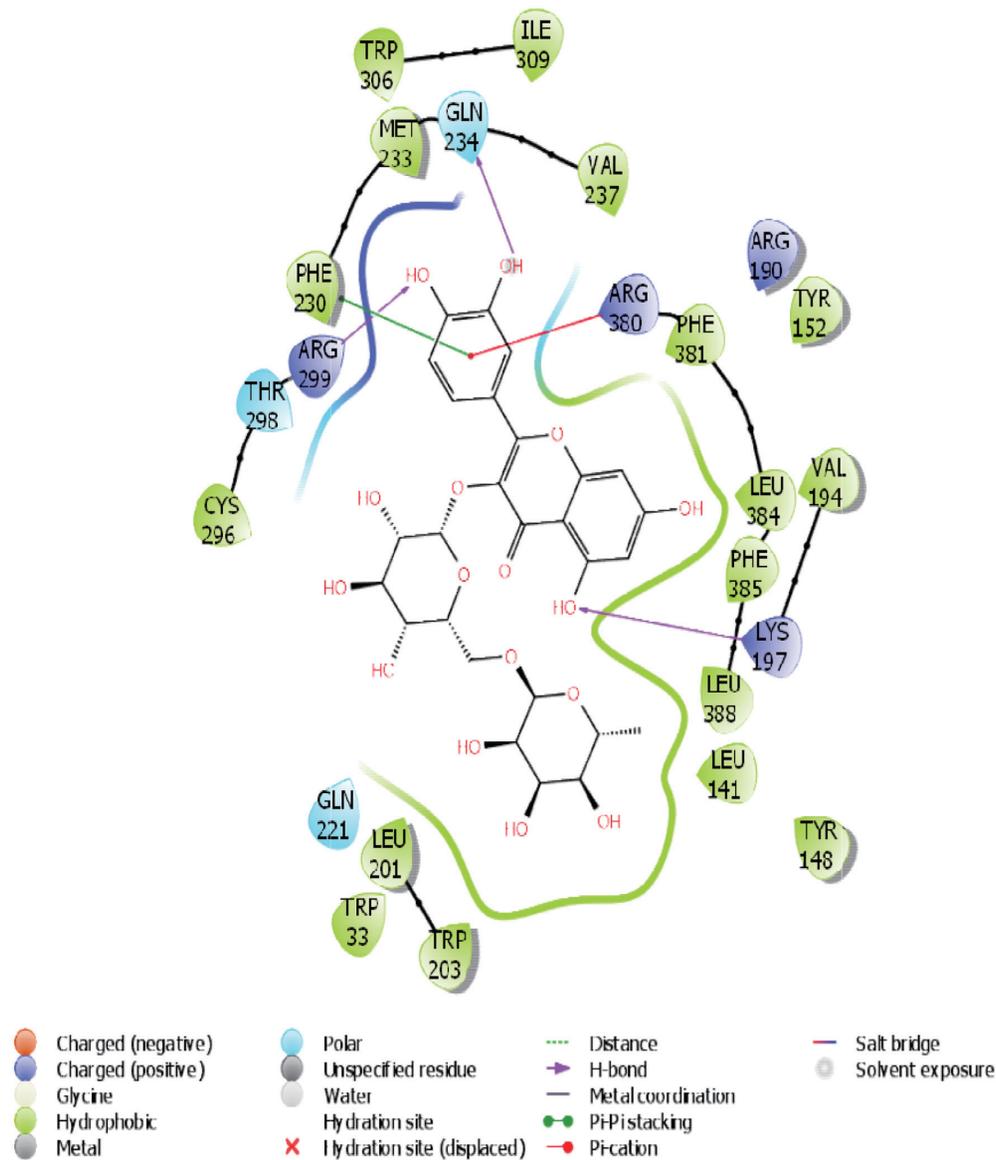


Figure 15. The 2D chemical relationship of Rutin with GLP-1 with amino acid residues.

of these cytokine genes relative to that in diabetic control rats in the pancreas (Figures 4 and 5). This could be due to the ability of rutin to reduce the amount of reactive oxygen species in the pancreas; this finding revealed the anti-inflammatory effect of rutin (Staurengho-Ferrari et al., 2019).

The expression of IL-1 β , IL-6 and TNF- α were studied in the liver as seen in Figures 6–8 respectively. The upregulation of these cytokines in the liver observed in the diabetic group could be a consequence of caspase-3 induction. This is an important enzyme in the apoptotic pathway that causes the death of hepatocytes (liver cells) resulting in hyperglycemia as liver play important role in glucose homeostasis (Faloon et al., 2011). Rutin (25, 50 and 100 mg/kg) significantly downregulated the expressions of these cytokines relative to diabetic control in the liver. This suggests that rutin has hepatoprotective potential.

TGR5 is a membrane-found G-protein-coupled receptor, and its activation induces GLP-1 release in endocrine cells (Javed et

al., 2012; Maruthur et al., 2016). The expression of TGR5 in diabetic rats was significantly lower than that in control rats (Figure 9). This decrease in TGR5 mRNA expression might be due to insulin resistance and the high glucose and/or fatty acid levels usually observed in diabetic patients. Insulin resistance and mitochondrial degeneration have been linked (Hunt et al., 2020). Rutin (50 and 100 mg/kg) significantly ($p < 0.05$) upregulated TGR5 expression relative to that in the diabetic control group. This could be due to rutin preventing oxidative stress and lipid accumulation. Glucagon-like peptide 1 (GLP-1) helps enhance pancreatic β -cell proliferation, boosts insulin production, and protects β -cells against apoptosis (Alonge et al., 2024). As shown in Figure 10, GLP-1 expression was significantly lower ($p < 0.05$) in the diabetic control group than in the control group. This could be a result of the death of beta cells (a source of the GLP-1 gene) caused by diabetes-induced inflammation. The oral administration of metformin and rutin at 25 and 100 mg/kg significantly upregulated (p

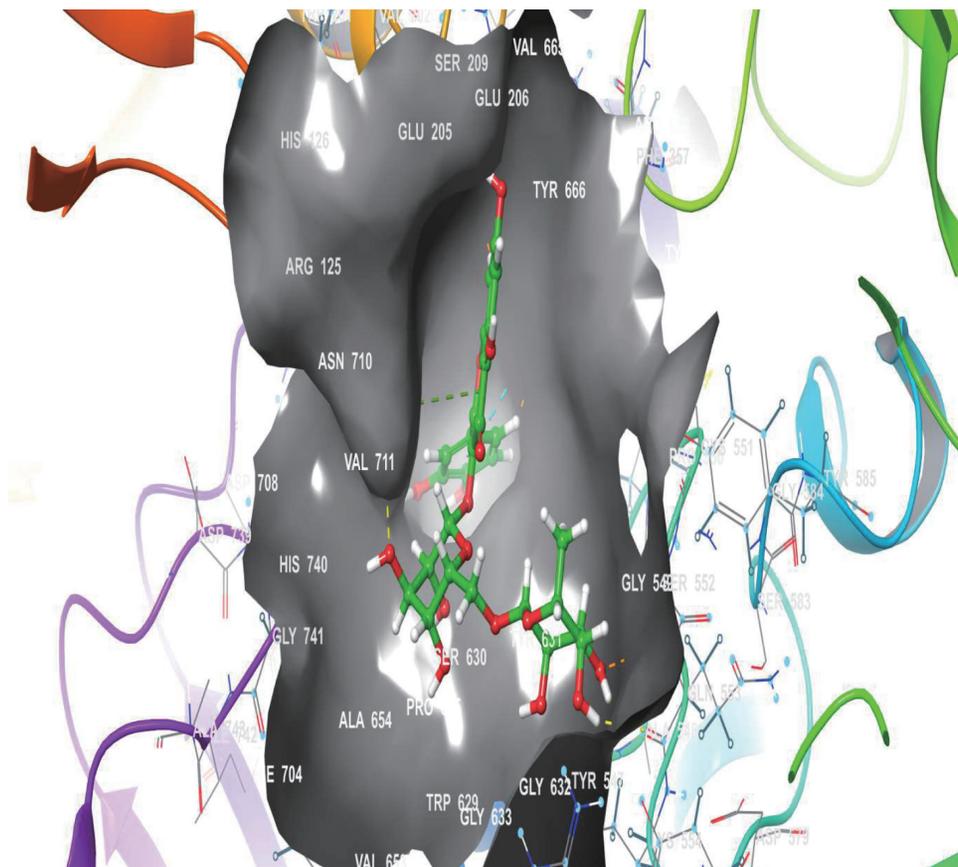


Figure 16. The 3D chemical relationship of Rutin with DPP4 with amino acid residues.

< 0.05) GLP-1 expression relative to that in the diabetic control group. This could be a result of the resuscitative effect of rutin on the pancreas leading to the activation and proliferation of beta cells. This finding is in agreement with the work of Oyetayo et al. (2021) who reported that flavonoids can boost the production of pancreatic beta cells.

As a peptidase, Dipeptidyl peptidase-4 (DPP4) hydrolyses and cleaves the incretin hormone GLP-1, which helps to increase excess glucose in the blood by converting it to glycogen (Elekofehinti et al., 2018). DPP4 expression was significantly upregulated in diabetic control rats compared to nondiabetic control rats in this study (Figure 11). This impairs glucose metabolism, resulting in increased blood sugar levels, as observed in this study. A half-life-shortening effect of DPP4 on hormones that ensure glucose homeostasis has been reported (Omar et al., 2014; Oyetayo et al., 2021). Rutin (25, 50, and 100 mg/kg) significantly downregulated the expression of DPP4 gene relative to that seen diabetic control group. This suggests that rutin may help to boost the actions of hormones that ensure euglycemia.

To further investigate the antidiabetic effect of rutin, the three-dimensional structures of TGR-5, GLP-1, and DPP4 were docked into the Rutin structure or compound. The docking results (Table 2) showed that TGR-5 and GLP-1 were highly correlated, with binding energies of -12.407 kcal/mol and -12.723 kcal/mol, respectively, when docked with the Rutin ligand (Figures 12–15). During the docking simulation of DPP4 with rutin, six H-bond formulations and 7 residues (GLN 553, SER 630, ARG 125, HIS 740, TYR 666, GLU 205) contributed to the hydrophobic interactions

around the active site (Table 2, Figures 16 and 17). The outcome of this *in silico* study suggests that rutin is a potent ligand of these receptors and capable of modulating them for its normoglycemic activity as seen in the glucose and gene expression results. This finding could further affirm the potential of rutin to exert its anti-inflammatory and antidiabetic effects, as shown in this study.

5. Conclusion

This study further affirms and supports the use of rutin in diabetes management as it reduced the blood glucose levels of diabetic rats after 28 days. Rutin also modulated the expressions of insulin-related genes and downregulated the expression of proinflammatory genes in the liver and pancreas of diabetic rats. It effectively binds to insulin-sensitive receptors. The work suggests that the most potent dosage of rutin in diabetes treatment is a low dosage.

Additional genes associated with glucose metabolism and inflammation may still be studied in rutin-administered diabetic rats. More research on various rutin doses could still be conducted to establish the most effective dosage and possibly clinical trials.

Acknowledgments

The authors appreciate the staff of Teady Bioscience Laboratory, Akure, Ondo State, Nigeria for their support and use of their fa-

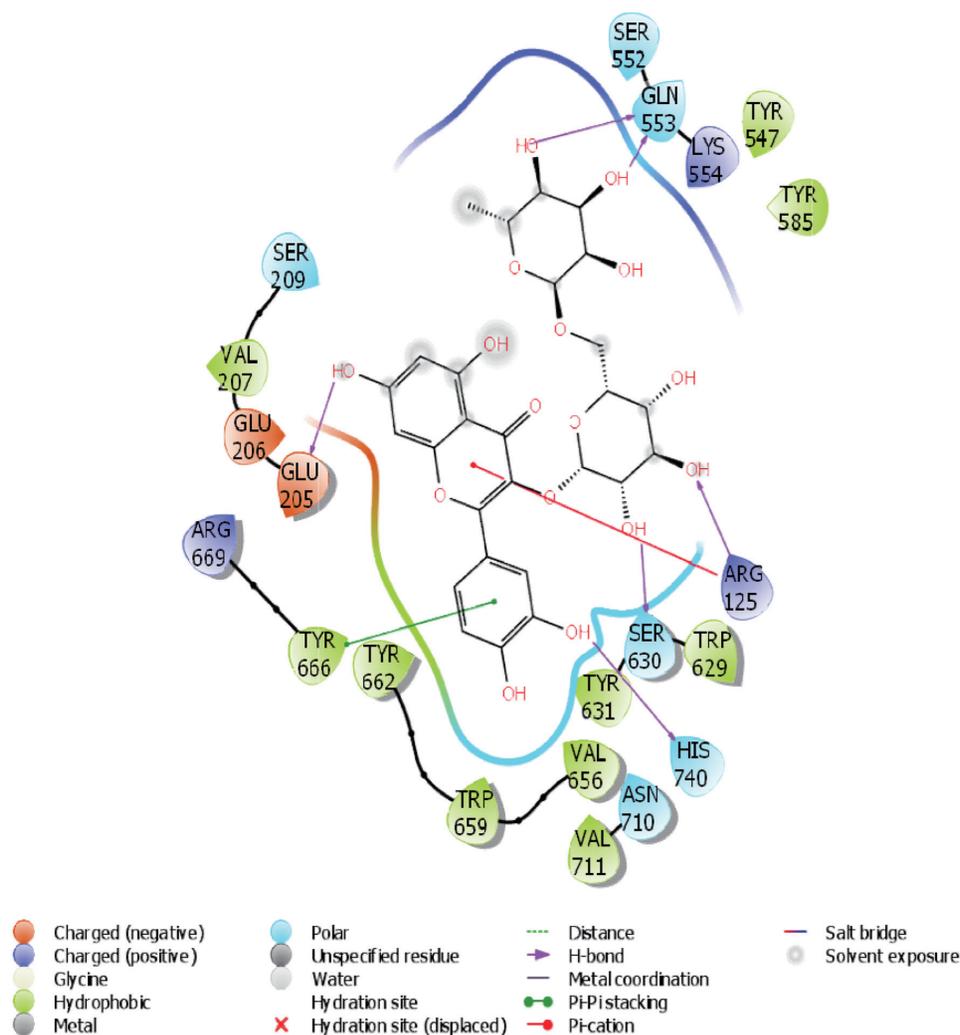


Figure 17. The 2D chemical relationship of Rutin with DPP4 with amino acid residues.

cilities for the study.

FUTA/ETH/24/146. Every procedure also followed the 1964 Declaration of Helsinki and its subsequent amendments

Ethical approval

Animal protocols were approved by the ethical committee of Federal University of Technology, Akure with the protocol number

Data availability

It will be made available on reasonable request

Table 2. Binding affinity, H-bonding, and hydrophobic interactions of rutin with selected receptor targets

No	Receptors and Rutin	Hydrogen Bonding	No of Pi-Pi links	Docking score (kcal/mol)	Binding Free Energy(kcal/mol)	Hydrophobic Interaction
1	GLP-1	3 (LYS 197, ARG 299, GLN 234)	1	-12.723	-41.36	17 (TRP 306, ILE 309, MET 233, VAL 237, CYS 296...PHE 230)
2	TGR-5	7 (SER 277, PHE 234, ILE 232, ILE 232, ARG 150, LEU 318, PRO 194)	0	-12.407	-42.87	13 (VAL 276, PHE 278, PHE 234, CYS 233, ILE 232 ...PRO 236)
3	DPP4	6 (HIS 740, ARG 125, GLN 553, GLN 553, SER 630, GLU 205)	1	-10.218	-50.58	10 (VAL 207, TYR 666, TYR 662, TRP 659, TYR 631, VAL 656, VAL 711, TRP 629, TYR 585, TYR 547)

Funding

No funding was received for this work

Conflict of interest

The authors declare that they have no known competing financial interests or personal interest

References

- Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Rep.* 50(3): 111–116.
- Ahrens, B. (2011). Antibodies in metabolic diseases. *N. Biotechnol.* 28: 530–537.
- Alonge, S.A., Elekofehinti, O.O., Akinjiyan, M.O., and Ajayi, I.I. (2024). Exploring the therapeutic mechanisms of *Astonia boonei* in diabetes mellitus ligand-based virtual screening with TGR5 Receptor. *J. Food Bioact.* 26: 61–67.
- Anderson, A.J., Jackson, T.D., Stroud, D.A., and Stojanovski, D. (2019). Mitochondria-hubs for regulating cellular biochemistry: emerging concepts and networks. *Open Biol.* 9(8): 190126.
- Arowosegbe, M.A., Amusan, O.T., Adeola, S.A., Adu, O.B., Akinola, I.A., Ogungbe, B.F., Omotuyi, O.I., Saibu, G.M., Ogunleye, A.J., Kanmodi, R.I., Lugbe, N.E., Ogunmola, O.J., Ajayi, D.C., Ogun, S.O., Oyende, F.O., Bello, A.O., Ishola, P.G., and Obasieke, P.E. (2020). Kaempferol as a potential PAK4 inhibitor in triple negative breast cancer: Extra precision glide docking and free energy calculation. *Curr. Drug Discovery Technol.* 17(5): 682–695.
- Butler, P.C., Meier, J.J., Butler, A.E., and Bhushan, A. (2007). The replication of beta cells in normal physiology, in disease and for therapy. *Nat. Clin. Pract. Endocrinol. Metab.* 3: 758–768.
- Cai, Z., Yuan, S., Zhong, Y., Deng, L., Li, J., Tan, X., and Feng, J. (2021). Amelioration of Endothelial Dysfunction in Diabetes: Role of Takeda G Protein-Coupled Receptor 5. *Front. Pharmacol.* 12: 637051.
- Deshpande, O.A., and Mohiuddin, S.S. (2021). *Biochemistry, Oxidative Phosphorylation*. StatPearls. StatPearls Publishing, Treasure Island (FL), Available from: <https://pubmed.ncbi.nlm.nih.gov/31985985/>.
- Edgerton, D.S., Moore, M.C., Gregory, J.M., Kraft, G., and Cherrington, A.D. (2021). Importance of the route of insulin delivery to its control of glucose metabolism. *Am. J. Physiol.: Endocrinol. Metab.* 320(5): E891–E897.
- Elekofehinti, O.O., Adetoyi, I.R., Popoola, H.O., Ayodeji, F.O., Taiwo, F.A., Akinjiyan, M.O., Koledoye, O.F., Iwaloye, O., and Adegboyega, A.E. (2024). Discovery of potential epidermal growth factor receptor inhibitors from black pepper for the treatment of lung cancer: an in-silico approach. *In Silico Pharmacol.* 12(1): 28.
- Elekofehinti, O.O., and Akinjiyan, M.O. (2020). Effects of *Momordica charantia* Silver Nanoparticles on Genes Associated with Lipid Metabolism and Nephrotoxicity in Streptozotocin-Induced Diabetic Rats. *Nig. J. Biotech* 37(2): 126–133.
- Elekofehinti, O.O., Ariyo, E.O., Akinjiyan, M.O., Olayeriju, O.S., Lawal, A.O., Adanlowo, I.G., and Rocha, J.B.T. (2018). Potential use of bitter melon (*Momordica charantia*) derived compounds as antidiabetics: in silico and in vivo studies. *Pathophysiology* 945: 1–7.
- Faloon, P.W., Chou, D.H., Forbeck, E.M., Walpita, D., Morgan, B., and Buhrhage, S. (2011). Identification of Small Molecule Inhibitors that Suppress Cytokine-Induced Apoptosis in Human Pancreatic Islet Cells. Probe Reports from the NIH Molecular Libraries Program [Internet]. National Center for Biotechnology Information (US), Bethesda (MD), Available from: <https://www.ncbi.nlm.nih.gov/books/NBK65431>.
- Ghasemi, A., and Jeddi, S. (2023). Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. *EXCLI J.* 22: 274–294.
- Ghorbani, A., Rashidi, R., and Shafiee-Nick, R. (2019). Flavonoids for preserving pancreatic beta cell survival and function: A mechanistic review. *Biomed. Pharmacother.* 111: 947–957.
- Hunt, J.E., Billeschou, A., Windeløv, J.A., Hartmann, B., Ullmer, C., Holst, J.J., and Kissow, H. (2020). Pharmacological activation of TGR5 promotes intestinal growth via a GLP-2-dependent pathway in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 318(5): G980–G987.
- Ijatuyi, T.T., Lawal, A.O., Akinjiyan, M.O., Ojo, F.M., Koledoye, O.F., Agboola, O.O., Dahunsi, D.T., Folorunso, I.M., and Elekofehinti, O.O. (2024). Effects of *Bryophyllum pinnatum* on Dysfunctional Autophagy in Rats Lungs Exposed to Zinc Oxide Nanoparticles. *Int. Immunopharmacol.* 141: 113005.
- Iwaloye, O., Elekofehinti, O.O., Kikiowo, B., Fadipe, T.M., Akinjiyan, M.O., Ariyo, E.O., Aiyeku, O.O., and Adewumi, N.A. (2021). Discovery of Traditional Chinese Medicine Derived Compounds as Wild Type and Mutant *Plasmodium falciparum* Dihydrofolate Reductase Inhibitors: Induced Fit Docking and ADME Studies. *Curr. Drug Discovery Technol.* 18(4): 554–569.
- Javed, H., Khan, M.M., Ahmad, A., Vaibhav, K., Ahmad, M.E., Khan, A., Ashafaq, M., Islam, F., Siddiqui, M.S., Safhi, M.M., and Islam, F. (2012). Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. *Neuroscience* 17: 340–352.
- Kim, J., Wei, Y., and Sowers, J.R. (2008). Role of Mitochondrial Dysfunction in Insulin Resistance. *Circ. Res.* 102: 401–414.
- Kim, S.K., and Hebrok, M. (2001). Intercellular signals regulating pancreas development and function. *Genes Dev.* 15: 111–127.
- Liang, N., Li, Y.M., He, Z., Hao, W., Zhao, Y., Liu, J., Zhu, H., Kwek, E., Ma, K.Y., He, W.S., and Chen, Z.Y. (2021). Rutin and Quercetin Decrease Cholesterol in HepG2 Cells but Not Plasma Cholesterol in Hamsters by Oral Administration. *Molecules (Basel, Switzerland)* 26(12): 3766.
- Lim, S., Bae, J.H., Kwon, H.S., and Nauck, M.A. (2021). COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat. Rev. Endocrinol.* 17(1): 11–30.
- Mahmoud, A.M., Hernández Bautista, R.J., Sandhu, M.A., and Hussein, O.E. (2019). Beneficial Effects of Citrus Flavonoids on Cardiovascular and Metabolic Health. *Oxid. Med. Cell. Longev.* 2019: 5484138.
- Maruthur, N.M., Tseng, E., Hutfless, S., Wilson, L.M., Suarez-Cuervo, C., Berger, Z., Chu, Y., Lyoha, E., Segal, J.B., and Bolen, S. (2016). Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. *Ann. Intern. Med.* 164: 740–51.
- Mutha, R.E., Tatiya, A.U., and Surana, S.J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future J. Pharm. Sci.* 7(1): 25.
- Nahdi, A.T., Arwa, M.T., John, A., and Raza, H. (2017). Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in rin-5F pancreatic β -cells. *Oxid. Med. Cell. Longev.* 20: 15.
- Nakrani, M.N., Wineland, R.H., and Anjum, F. (2023). *Physiology, Glucose Metabolism*. StatPearls. StatPearls Publishing.
- Navale, A.M., and Paranjape, A.N. (2016). Glucose transporters: physiological and pathological roles. *Biophys. Rev.* 8(1): 5–9.
- Ojo, O.A., Ibrahim, H.S., Rotimi, D.E., Ogunlakin, A.D., and Ojo, A.B. (2023). Diabetes mellitus: From molecular mechanism to pathophysiology and pharmacology. *Med. Nov. Technol. Devices* 19: 100247.
- Oloyede, O.I., Elekofehinti, O.O., Akinjiyan, M.O., Ajayi, E.B., Coker, F., and Olabinjo, I.A. (2022). Effect of *Cymbopogon citratus* (Lemon grass) on the Expression of Insulin sensitive and Proinflammatory Genes in the Pancreas of Diabetic Rats. *Diabetes Obes. Int. J.* 7(3): 000258.
- Omar, B.A., Liehua, L., Yamada, Y., Seino, Y., Marchetti, P., and Ahrén, B. (2014). Dipeptidyl peptidase 4 (DPP-4) is expressed in mouse and human islets and its activity is decreased in human islets from individuals with type 2 diabetes. *Diabetologia* 57(9): 1876–1883.
- Oyetayo, F.L., Akomolafe, S.F., Jegede, F.O., Elekofehinti, O.O., Akinjiyan, M.O., and Odeniyi, I.A. (2021). Effect of *Chrysophyllum albidum* fruit pulp powder on antioxidant and proinflammatory genes in non-diabetic and type 2 diabetic rats. *J. Diabetes Metab. Disord.* 20(2): 1663–1674.
- Perez-Matute, P., Zulet, M.A., and Martinez, J.A. (2009). Reactive species and diabetes: counteracting oxidative stress to improve health. *Curr. Opin. Pharmacol.* 9: 771–779.
- Siddique, T., and Awan, F.R. (2016). Effects of Reg3 Delta Bioactive Peptide on Blood Glucose Levels and Pancreatic Gene Expression in an Alloxan-Induced Mouse Model of Diabetes. *Can. J. Diabetes* 40(3): 113–120.

- 198–203.
- Song, S.O., Yun, J.S., Ko, S.H., Ahn, Y.B., Kim, B.Y., Kim, C.H., Jeon, J.Y., Kim, D.J., Seo, D.H., Kim, S.H., Noh, J.H., Lee, D.Y., Kim, K.S., Kim, S.K., and Type 1 Diabetes Study Group of Gyeonggi-Incheon Branch of the Korean Diabetes Association. (2022). Prevalence and clinical characteristics of fulminant type 1 diabetes mellitus in Korean adults: A multi-institutional joint research. *J. Diabetes Invest.* 13(1): 47–53.
- Staurengo-Ferrari, L., Badaro-Garcia, S., Hohmann, M.S.N., Manchope, M.F., Zaninelli, T.H., Casagrande, R., and Verri, W.A. Jr. (2019). Contribution of Nrf2 Modulation to the Mechanism of Action of Analgesic and Anti-inflammatory Drugs in Pre-clinical and Clinical Stages. *Front. Pharmacol.* 9: 1536.
- Talib, W.H., Mahmud, A.I., Abuarab, S.F., Hasen, E., Munaim, A.A., Haif, S.K., and Kury, L.T.A. (2021). Diabetes and Cancer: Metabolic Association, Therapeutic Challenges, and the Role of Natural Products. *Molecules* 26(8): 2179.
- Wang, D., and Farhana, A. (2023). *Biochemistry, RNA Structure*. StatPearls Publishing.
- Wu, J., Jin, Z., and Yan, L.J. (2017). Redox imbalance and mitochondrial abnormalities in the diabetic lung. *Redox Biol.* 11: 51–59.
- Wu, J., Jin, Z., Zheng, H., and Yan, L.J. (2016). Sources and implications of NADH/NAD(+) redox imbalance in diabetes and its complications. *Diabetes Metab. Syndr. Obes.* 9: 145–153.
- Zhong, J., Gong, Q., and Mima, A. (2017). Inflammatory Regulation in Diabetes and Metabolic Dysfunction. *J. Diabetes Res.* 2017: 5165268.
- Zimmet, P., Alberti, K.G., Magliano, D.J., and Bennett, P.H. (2016). Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. *Nat. Rev. Endocrinol.* 12(10): 616–622.