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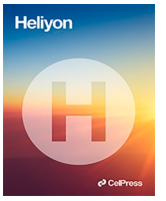

Research article

Protective activity of hirsutidin in high-fat intake and streptozotocin-induced diabetic rats: In silico and in vivo study

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is defined by a wide variety of metabolic abnormalities, persistent hyperglycemia, and a slew of other complications. *Catharanthus roseus* L. (apocyanaceae), remarkably notable as *Vinca Rosea*, appears to be the source of the active component hirsutidin, which is reported in various diseases.
Objective: The study intended to appraise the antidiabetic capability of hirsutidin in a high-fat diet (HFD) and streptozotocin (STZ) induced diabetes in experimental rats.
Materials and methods: An experimental rodent T2DM model was elicited by consuming an HFD regimen with STZ 50 mg/kg, i.p. dose formulated in a 0.1 M cold citrate buffer (pH 4.5). The test drug hirsutidin (10 and 20 mg/kg) and the standard drug glimeclamide (5 mg/kg) were administered daily for six weeks. The efficacy of hirsutidin was observed on several diabetes parameters. The average body weight and an array of biochemical markers were determined, including blood glucose, insulin, dyslipidemia (lipid profile), total protein (TP), liver injury [aspartate aminotransferase (AST), alanine aminotransferase (ALT)], inflammation [IL-6, IL-1β, tumor necrosis factor-α (TNF-α)], oxidative stress [malondialdehyde (MDA)] and antioxidant status [catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD)]. In addition, the concentrations of leptin, adiponectin, and resistin were also assessed. Also, molecular docking studies were undertaken to investigate critical targets associated with diabetes, including TNF-α, insulin, adiponectin, and leptin.
Results: Diabetes induction with HFD/STZ resulted in hyperglycemia (significantly reduced blood glucose and increased insulin level), dyslipidemia (significantly reduced TC, TG and increased HDL), total protein (significantly reduced), oxidative stress and antioxidant status (significantly

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reduced MDA and increased CAT, SOD and GSH levels), inflammation (significantly decreased IL-6, IL-1β, TNF-α), liver damage (significantly reduced AST, ALT), and specific hormones such as adiponectin, leptin significantly improved and resistin significantly reduced as evidenced by biochemical data in this study. Intermolecular interactions of ligands and docking score, hirsutidin proteins TNF-α (2AZ5), Insulin (4IBM), Adiponectin (6KS1), Leptin (7Z3Q) with binding energy of -6.708, -7.674, -7.2 and -7.547 Kcal/mol.

Conclusion: Hirsutidin may have an evidential hypoglycemic outcome and may exhibit potent antidiabetic activity in HFD/STZ-induced T2DM in rats. Treatment with hirsutidin significantly improved glycemic control, lipid metabolism, oxidative stress, inflammation, and liver function. Additionally, it normalized dysregulated levels of adiponectin, leptin, and resistin. Molecular docking confirmed its strong binding affinity to key diabetic targets.

1. Introduction

A wide variety of metabolic abnormalities, persistent hyperglycemia, and a slew of other complications define type 2 diabetes mellitus (T2DM) [1–4]. At present, DM affects 463 million people globally, and it is a matter, of course, to grow to 700 million by 2045 [5,6]. The overflowing prevalence of DM and its social and economic significance make it a global threat. The ingestion of high-fat foods contributes to obesity and the development of many diseases, including DM [7]. The potential methods by which this illness is mediated are yet unknown. Obesity is known to impair adipose tissue metabolic and endocrine function, resulting in an alteration in fatty acid synthesis, hormones, and proinflammatory chemicals, all of which contribute to obesity-related problems [8]. Obesity-related changes in adipokine secretion may play a dramatic role part in diet-induced diabetes [9–11]. Leptin and adiponectin, two major adipocytokines, are thought to play crucial parts in the dominance of cardiovascular and metabolic balance [12]. Enlarged adipocytes and monocyte-derived macrophages promote a pro-inflammatory state by increasing the production of inflammatory cytokines like TNF-α and IL-6 and decreasing the production of anti-inflammatory adiponectin [13]. TNF-α exhibits a positive correlation with both hyperinsulinemia and obesity. In contrast, in an obese insulin-resistant rodent model, an increase in adiponectin levels preceded the onset of diabetes. Adiponectin also has antidiabetic and insulin-sensitizing effects [14]. Attempts should be oriented towards treating both diseases as a whole, rather than separately, by developing improved therapies and innovative preventative techniques for T2DM co-occurring with a variety of comorbidities [15].

Animal models are used to research many aspects of the illness, such as its symptoms, evolution, and consequences [16]. This necessitates greater knowledge of the impact of environmental variables that impact illness transmission and intensity, such as dietary and lifestyle changes. A plethora of diabetes modeling approaches have been created. Among these methods, the creation of a diabetic model using streptozotocin (STZ) in conjunction with a high-fat diet (HFD) has been frequently employed [17,18]. Combining an HFD with STZ induces β-cell malfunction in animals [19]. A rising amount of animal research shows that STZ coupled with HFD can mimic many aspects of diet-induced T2DM found in people [20]. It has been proposed that the HFD may be a superior approach for processing insulin resistance, which is one of the fundamental features of T2DM. As a result, rat models fed a high-fat diet along with STZ were employed for research that closely mimicked the natural course of disease episodes and metabolic aspects of human T2DM [21].

T2DM is treated with a combination of diet, exercise, and the use of insulin and/or oral hypoglycemic medications. Exploring novel medication therapy might be unsuccessful against specific long-term diabetes problems while also lowering the high expense of health care. The World Health Organization (WHO) has advocated the sensible utilization of conventional and natural indigenous remedies for treating DM. Plants and their bioactive substances have recently become more widely used as supplementary therapies for T2DM. Approximately 80 % of the population with diabetes around the world relies on medicative herbs to help them manage their condition [22]. Hirsutidin is an anthocyanidin first discovered in *Catharanthus roseus* and belongs to the Apocyanaceae family. It has various pharmacological properties, including antioxidant, antiulcer, antibacterial, hypotensive, anticancer, anti-inflammatory, and antidiabetic properties [23]. It contains many more alkaloids, some of which are authorized as antineoplastic drugs for the treatment of diseases such as malignant lymphomas, leukemia, Hodgkin's disease, Wilms tumor, neuroblastoma, and others [24]. Although different advantages of hirsutidin have been described through various pathways [25–28], there is currently no experimental evidence in the literature regarding its HFD/STZ-caused diabetes activity. In this study, we investigated the role of hirsutidin in a type 2 diabetic model. Furthermore, based on a thorough analysis of the available literature, our research focused on critical targets related to diabetes, such as TNF-, insulin, adiponectin, and leptin.

2. Methods

2.1. Animals

This experimental research employed Wistar male rats weighing 180 ± 20g (n = 6, 10–12 weeks old). Wistar rats were kept in institutional animal facilities in poly acrylic cages with fewer than three rats per enclosure in air-conditioned environs with a light and dark cycle. The experimental rats were given free way to a conventional diet or an HFD, as needed, and water.

2.2. Chemicals and test drugs

STZ was received from Sigma-Aldrich, USA. HFD was procured from Trans-Genica Services Pvt. Ltd., M.S., India, and hirsutidin was obtained from MSW Pharma, M.S., India. Other consumables utilized were all analytical grades from an attested local vendor.

2.3. Experimental design

All the experimental rats studied were served an HFD containing 60 % of total calories until the completion of the paradigm, which was six weeks. Exploration was directed in agreement with ARRIVE's directive, with approval from the institution's animal ethical committee (TRS/PT/21/09/IAEC). Animals used in the study had no previous procedure. After dietary manipulation (2 weeks), on the 14th day of the investigation regime, the animals were fasted (12 h) and given a 50 mg/kg STZ i.p. dose formulated in a 0.1 M cold citrate buffer (pH 4.5). The medical aid commenced on the 14th day following the STZ installation, which was designated the commencement of the management design. It persisted for four weeks, using medication glibenclamide 5 mg/kg and hirsutidin 10 and 20 mg/kg/day p.o., respectively [27,29]. Hyperglycemic rats demarcated as 250 mg/dl blood sugar after one week of STZ instillation were classified as T2DM animals and considered in the investigation. Blood samples were obtained from the retroorbital plexus for biochemical parameter evaluation. Scarification of experimental animals was accomplished with phenobarbitone 60 mg/kg i.p. The present investigation design was based on the prior report [19].

All animals were simply randomized and allotted to five groups for one week following the adaptation phase. Group 1: Saline was given to the normal control group. Group 2: Disease group was given HFD/STZ. Diabetic rats in group 3 were given glibenclamide at 5 mg/kg body weight. Diabetic animals were administered 10 mg/kg and 20 mg/kg of hirsutidin in groups 4 and 5, respectively. At the end of study blood was composed in centrifuge tubes without anticoagulant, permitted to clot, and then centrifuged at 3000 rpm for 20 min. The serum was kept at 20 °C till employed in biochemical investigations. Using a Potter Elvehjem homogenizer, the liver sample was homogenized (10 % w/v) in frozen 0.1 M Tris-HCl buffers (Sigma Aldrich, USA). The homogenate was centrifuged for 15 mins at 4 °C with an acceleration of 860g, and the supernatant was applied to various assays.

2.4. Evaluation parameters

Body weight: On a weekly basis, the body weight was recorded using a calibrated weighing balance.

Blood glucose: The glucometer was used to monitor blood glucose once a week (Accu-Check, Roche, Germany).

Basic blood biochemistry: Serum lipid contour (high-density lipoprotein-HDL, total cholesterol-TC and triglycerides-TG), as well as hepatic enzymes (alanine transaminase-AST and aspartate transaminase-ALT), were assessed in agreement with the manufacturer's recommendations. The parameters were measured using a reader in accordance with the manufacturer's guide and enzymatic kits [30].

Inflammatory markers: Tumor necrosis factor alpha (TNF-α) and Interleukins (IL)-1β, IL-6 serum concentrations were dictated using an ELISA technique [31].

Oxidative status: Previously published study methods with minor modifications were used to estimate serum oxidative stress indicators. Lowry et al. provided a technique for assessing concentrations of protein [32]. Colorimetric measurement of catalase (CAT) enzyme activity in the occurrence of glacial acidic acid and hydrogen peroxide at 570 nM is one of Sinha's previously disclosed methods for determining CAT enzyme activity [33]. Via spectrophotometric approach, the pink chromogen designed after relating with thiobarbituric acid reactive substance, which reveals the formation of malondialdehyde (MDA) as the outcome of the lipid peroxidation operation, was detected at 532 nM [34]. Similarly, the determination of reduced glutathione (GSH) generated a yellow-coloured molecule on a spectrophotometric valuation using commercially accessible kits at 405 nM [35]. The superoxide dismutase (SOD) action was premeditated by a 96-well plate reader set to 490 nM, commercial supplies, and the manufacturer's exercise to calculate the extent of protein essential to prevent 6-hydroxydopamine auto-oxidation [36]. Further, protein was estimated by adding 10 μl tissue supernatant to 100 μl of 1 m-NaOH and 1 ml Bradford reagent. The obtained solution was vortexed, and the

Table 1
Comparing the recovered protein with standard values to validate the protein chosen for docking investigation.

Parameters	Protein particulars				Standards
Targets	**Tnf-α**	**Insulin**	**Adiponectin**	**leptin**	–
Protein id	**2AZ5**	**4IBM**	**6KS1**	**7Z3Q**	–
Method of experiment	X-ray diffraction	X-ray diffraction	X-ray diffraction	X-ray diffraction	X-ray diffraction
Mutation	No	No	No	No	No
Resolution	2.10 Å	1.80 Å	2.40 Å	3.62 Å	Near about 2.00 a^0
Wwpdb validation	Better	Better	Better	Better	Better
Co-crystal ligand	307	Ir1	Absent	Absent	–
Ramchandran plot (by procheck server)Residues in favoured + allowed regions	90.2 %	92.5 %	93.6 %	92.2 %	>80 %

Residues in favoured + allowed regions

absorbance was recorded at 595 nm [37].

Estimation of adiponectin, leptin, resistin, and insulin: Serum levels of leptin, resistin, adiponectin and insulin were dictated by ELISA tools using the previously indicated methodologies and procedures with minor modifications recommended by the manufacturers.

2.5. Molecular docking studies

2.5.1. Target protein retrieval and preparation

Based on a comprehensive review of existing works, our research focused on investigating critical targets associated with diabetes, including TNF-α, insulin, adiponectin, and leptin (Table 1). To do this, we retrieved the FASTA sequences of these proteins for the *Homo sapiens* taxon from the National Center for Biotechnology Information (NCBI) server. Afterward, we searched for similar biological sequences in the Protein Data Bank (PDB) using the Basic Local Alignment Search Tool (BLAST). We carefully selected the top 5 to 10 orders based on factors such as E-value, percentage identity and query coverage.

Next, we obtained the three-dimensional X-ray crystallographic structures of TNF-α, insulin, adiponectin, and leptin from the PDB database using their respective accession numbers. These structures were validated through numerous considerations, comprising resolution, mutation analysis, wwPDB confirmation, the presence of co-crystal ligands, and assessment of the Ramachandran plot.

Before conducting docking studies, optimizing and minimizing the molecules, whether they are macromolecules or micromolecules, is crucial. To ensure the accuracy of our research, we first verified the residues within the binding pockets of the proteins using the PDBsum server. PDBsum is a visual catalogue of 3D assemblies in the Protein Data Panel that provides information about interactions between standard inhibitors and proteins.

We addressed missing residues and generated side chains using CHIMERA v1.16 to prepare the proteins for docking studies. Subsequently, optimization and minimization procedures were applied. The optimization process involved 1000 Steepest Descent steps with a Steepest Descent size of 0.1 Å, monitored by 100 conjugate gradient steps with a size of 0.1 Å. Additionally, we ensured that all hydrogen atoms, including the slower ones, were added and protonation positions were set for histidine. Concerns were incorporated using the AMBER ff14SB force field. The optimization and minimization of nonstandard remains, such as water molecules and excessive chains, were removed from the proteins using V21.1.0.20298 Biovia Discovery Studio Visualizer.

2.5.2. Grid compeers

Three programs were used to identify receptor grids: AutoDockTools, Chimera, and Maestro. The Workspace presented the prepared proteins (2AZ5, 4IBM 6KS1 and 7Z3Q). The volume of the grid for proteins with co-crystal ligands was determined using the dimensions of the ligand, and for proteins without co-crystal ligands, the volume of the grid was computed using the CASTp server (Computed Atlas of Surface Topography of Proteins). The information about amino acids is found in the grid pocket (Table 2). The enclosing case was made tiny to maintain consistency with the expected docking of ligands and the structure and characteristics of the protein's active site.

2.5.3. Ligands preparation

Targeted Molecule imported in MarvinSketch v21.13, cleaned in 2D and 3D formats, minimized using the MMFF94 force field, and chosen lowest energy conformer, which is then protected in 3D mol2 file format.

2.5.4. Statistical analysis

The data was considered by Windows-based software (GraphPad Prism; Version 8.0.2). The current study findings are provided as Mean ± SEM. One-way investigation of alteration monitored by Turkey's numerous contrast tests was used to determine the evidential points and show how the variables differed across the groups. $P < 0.05$ was considered as significant.

Table 2
Active sites amino acids.

PROTEIN	ACTIVE SITES AMINO ACIDS
2AZ5	LEU57A, TYR59A, SER60A, TYR119A, LEU120A, GLY121A, GLY122A, TYR151A, LEU57B, TYR59B, SER60B, TYR119B, LEU120B, GLY121B, TYR151B, LEU55D
4IBM	ALA1121, ASN1124, ALA1125, LYS1127, PHE1128, VAL1129, PRO1178, GLU1179, LYS1182, ASP1183, VAL1185, THR1187, THR1188, SER1189, MET1192, MET1223, ASP1224, GLY1225, PHE1248, ASN1249, PRO1250, ASN1251, ARG1253, PRO1254, THR1255, PHE1256, LEU1257, GLU1258, PHE1276, GLU1281, ASN1282, GLN1004, GLY1005, SER1006, PHE1007, GLY1008, VAL1010, LYS1030, THR1031, ARG1039, GLU1040, ILE1042, GLU1043, PHE1044, ASN1046, GLU1047, ALA1048, VAL1050, MET1051, VAL1074, LEU1123, HIS1130, ARG1131, ASP1132, ARG1136, ASN1137, MET1139, GLY1149, ASP1150, PHE1151, GLY1152, MET1153, THR1154, ARG1155, ASP1156, ILE1157, GLU1159, TYR1162, ARG1164, LYS1168, GLY1169, LEU1170, LEU1171, PRO1172, MET1176, PHE1186, LYS1283
6KS1	THR142, VAL145, ALA146, ARG149, ILE85, MET89, ASP106, HIS109, VAL110, PHE113, VAL22, SER470, LEU471, TYR472
7Z3Q	HIS108, LEU111, ASP113, TRP114, LEU115, LYS116, ASP117, ASN118, LEU121, HIS125, ARG126, HIS143, THR144, GLU145, ASN148, HIS152, PHE187, CYS194, SER198, PHE201, HIS202, TYR205, CYS206, GLU209, SER212, ARG213, SER216, LYS217, ASP219, TYR220, ILE223, LEU226, ILE227, SER230, PHE231, TRP234, SER264, TRP266, ASP267, ALA270, THR271, PRO272, ARG275, ARG278, ALA279, PHE282, LEU283, GLY286, LEU287, GLY289, ILE290, THR293, ILE308, GLY309, GLN310, TRP313, LEU314, MET317, ALA318, LEU320, TYR321, GLY324, ALA325, TYR328, ARG331, GLU334, HIS346, SER347, HIS348, PHE351, HIS352, PHE354, VAL355, GLY358, ALA359, VAL361, HIS362, GLY365, VAL366, ASN368, LEU369

3. Results

3.1. Effects of hirsutidin on body weight

The body weights of the research rats are shown in Fig. 1. In contrast to the STZ/HFD-caused type 2 diabetic group, the conventional treatment of glibenclamide 5 mg/kg treated group and hirsutidin treated groups of 10 and 20 mg/kg considerably recovered the animals' body weight ($P < 0.05$). The standard and test groups do not differ statistically significantly [$F(4, 50) = 0.04101$; $P = 0.9967$].

3.2. Effects of hirsutidin on blood glucose and insulin

Studies on diabetes emphasize finding appropriate management for hyperglycemia as their top priority. As a result, hirsutidin had hypoglycemic properties in diabetic animals. As demonstrated in Fig. 2, in contrast to the normal control group, fasting blood glucose was elevated following induction with HFD/STZ on day 21 and persisted high throughout the trial, indicating efficient diabetes development in all animals compared to normal control rats. After four weeks of treatment, the test drug hirsutidin 20 mg/kg and conventional drug glibenclamide 5 mg/kg exhibited an extremely evidential reduction in blood glucose, although hirsutidin 10 mg/kg was shown to be somewhat rationally effective [$F(4, 125) = 61.56$; $P < 0.0001$]. Furthermore, the experimental investigation demonstrated a substantial drop in insulin levels in animals with diabetes compared to saline-treated rats. In comparison to the HFD/STZ disease control animals, the test drug hirsutidin (10 and 20 mg/kg) and the standard medicine glibenclamide (5 mg/kg) recovered reduced insulin [$F(4, 25) = 8.768$; $P = 0.0001$] (Fig. 3).

3.3. Effects of hirsutidin on lipid metabolism and total protein

Directly compared to the ailment rats, hyperlipidemia was demonstrated following the induction of HFD/STZ by a considerable fall

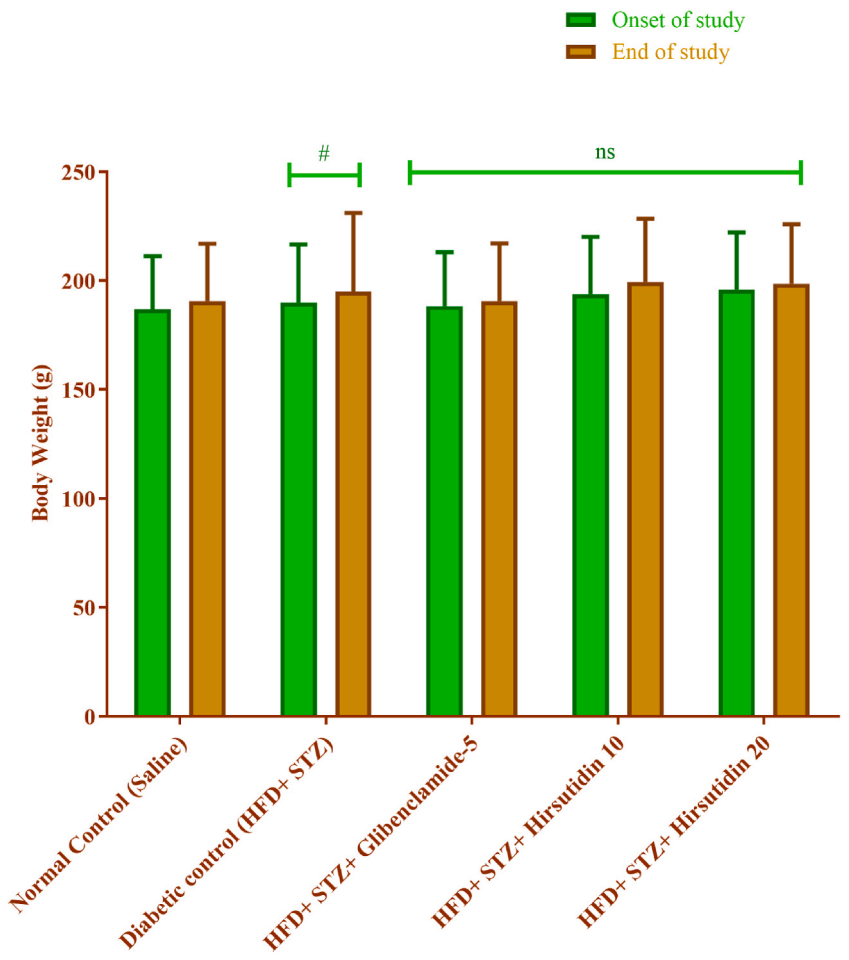

Fig. 1. Outcome of hirsutidin on mean body weight. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ, ns-not significant.

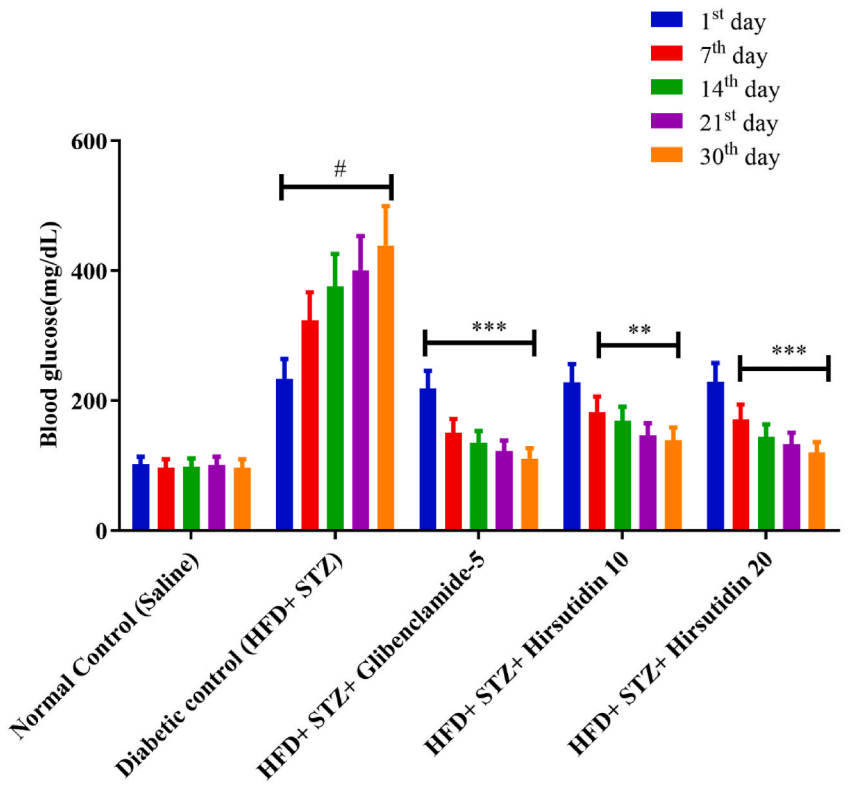

Fig. 2. Outcome of hirsutidin on blood glucose in HFD/STZ-induced diabetic rats. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; $^{**}P < 0.001$, $^{***}P < 0.0001$ Vs HFD + STZ.

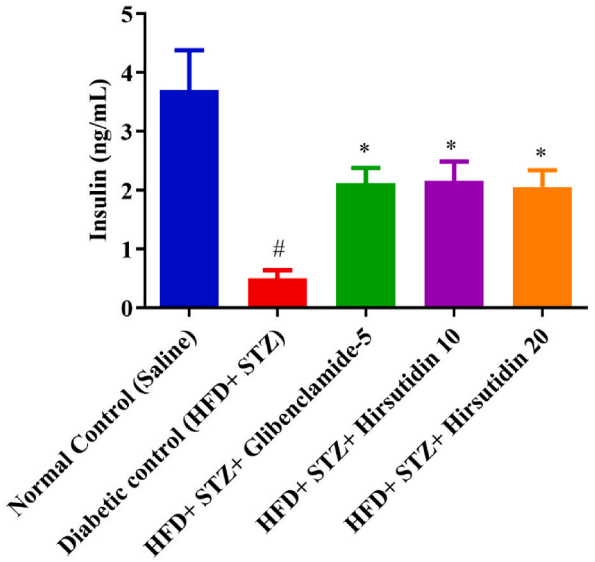

Fig. 3. Outcome of hirsutidin on insulin in HFD/STZ-induced diabetic rats. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; $^{*}P < 0.05$ Vs HFD + STZ.

in HDL and gain in blood TG and TC. In contrast to the untreated cluster, hirsutidin 10 and 20 mg/kg and the standard medication glibenclamide 5 mg/kg in HFD/STZ-induced animals resulted in a fundamental reduction in TG [$F(4, 25) = 7.145$; $P = 0.0006$] and TC [$F(4, 25) = 11.55$; $P < 0.0001$] as well as a considerable increase in HDL [$F(4, 25) = 6.087$; $P = 0.0015$]. Moreover, treatment with both doses of hirsutidin in rats had superior results, with a more substantial drop in lipid contour. Plasma protein level reductions have been linked to HFD/STZ-evoked diabetes. Disease control animals showed a decrease in protein levels, which were restored after

management with tests and standard [F (4, 25) = 9.593; P < 0.0001] group due to glucose management (Fig. 4A–C, Fig. 5).

3.4. Effects of hirsutidin on adiponectin, resistin and leptin

Outcome of hirsutidin on leptin, adiponectin and resistin in HFD/STZ-induced diabetic animals. The results of the study demonstrated a significant reduction of adiponectin and leptin in the HFD/STZ group. However, resistin levels increased significantly compared to normal rats. HFD/STZ-induced animals were given the accepted medication glibenclamide (5 mg/kg) and hirsutidin (10 and 20 mg/kg), which resulted in a considerable upsurge in leptin [F (4, 25) = 4.389; P = 0.0080] and adiponectin [F (4, 25) = 7.039; P = 0.0006] levels related to the disease rats, but an evidentiary decrease in resistin [F (4, 25) = 8.937; P = 0.0001] after treatment related to the disease animals. The hirsutidin effectively responded to leptin, adiponectin, and resistin (Fig. 6A–C).

3.5. Effect of hirsutidin on the oxidative biomarkers

The HFD/STZ-induced rats incontestable showed a substantial drop in intracellular SOD, GSH and CAT levels but a significant upsurge in MDA related to the normal control group. The treatment schedule that included both the formulaic glibenclamide 5 mg/kg and the hirsutidin (10 and 20 mg/kg) treatment substantially reduced intracellular indices of MDA [F (4, 25) = 8.319; P = 0.0002]. Moreover, it repairs the inhibited antioxidant enzymes CAT [F (4, 25) = 6.963; P = 0.0007], SOD [F (4, 25) = 4.266; P = 0.0091] and GSH [F (4, 25) = 6.411; P = 0.0011] in contrast to the HFD/STZ-induced group (Fig. 7A–D).

3.6. Effects of hirsutidin on proinflammatory cytokines

To determine the mechanism by which the hirsutidin has anti-infammatory potential in rats. Fig. 8 A-C shows the proinflammatory cytokines such as IL-6, TNF-α and IL-1β in an assorted study design. When TNF-α, IL-6 and IL-1β were examined in HFD/STZ-induced rats and the normal control group, there was an evidentiary rise in IL-1β, TNF-α and IL-6 in d HFD/STZ-induced rats. The TNF-α [F (4, 25) = 6.550; P = 0.0009], IL-1β [F (4, 25) = 10.34; P < 0.0001], and IL-6 [F (4, 25) = 13.96; P < 0.0001] was pronounced brought down in hirsutidin treatment rats and standard drug glibenclamide treatment compared to HFD/STZ-induced rats. Surprisingly, these increases in inflammatory markers were significantly suppressed by hirsutidin treatment.

3.7. Effects of hirsutidin on the concentration of AST and ALT

The results indicated that HFD/STZ significantly increased AST and ALT hepatic biomarkers in the HFD/STZ-induced rats. In relation to the diabetic rats, standard drug and hirsutidin (10 and 20 mg/kg) significantly lowered ALT [F (4, 25) = 8.303; P = 0.0002] and AST [F (4, 25) = 14.70; P < 0.0001] levels (Fig. 9A–B).

3.8. Molecular docking of target protein with ligands

Following the acquisition of the protein and ligand, the assemblies of both were transformed to the pdbqt design by an internal bash script created using the AutoDock tools 1.5.7 for ligands and ADFRsuit for proteins, in which all the rotatable connections of the ligands were permitted to rotate freely and the receptor were deliberated rigid. We utilized the AutoDock Vina 1.2.5 with a grid layout of 0.375 A for docking investigations. The grid case was placed on the target's dynamic site, enabling the software to look for other potential sites of ligand and receptor interactions. Other configurations were considered default. The grid box's dimensions and XYZ coordinates are listed in Table 3. Other values, including CPU, were set to 23 and 32, respectively, there were 9 modes and a 3-energy

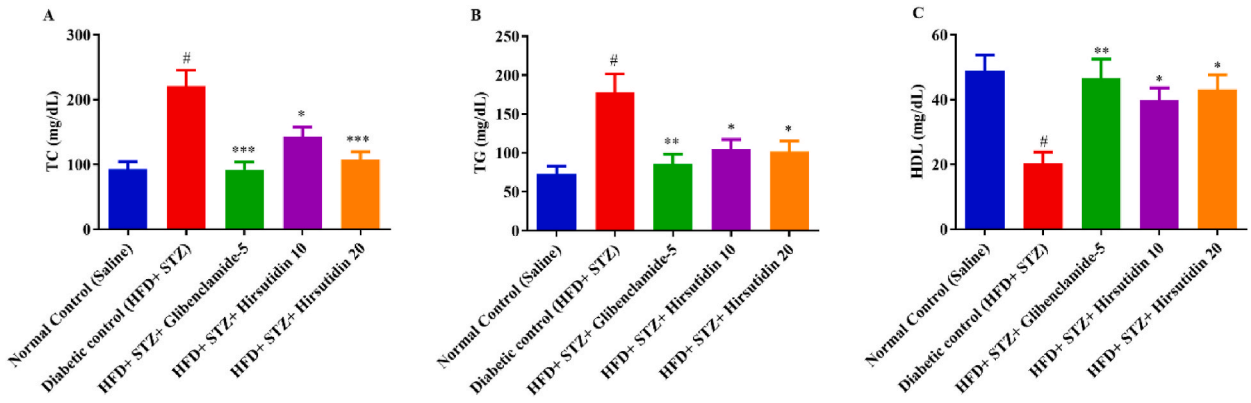

Fig. 4. A–C: Outcome of hirsutidin on lipid profile in HFD/STZ-induced diabetic rats. (A) TC: total cholesterol; (B) TG: triglycerides; (C) HDL: high-density lipoprotein. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; *P < 0.05, **P < 0.001, ***P < 0.0001 Vs HFD + STZ.

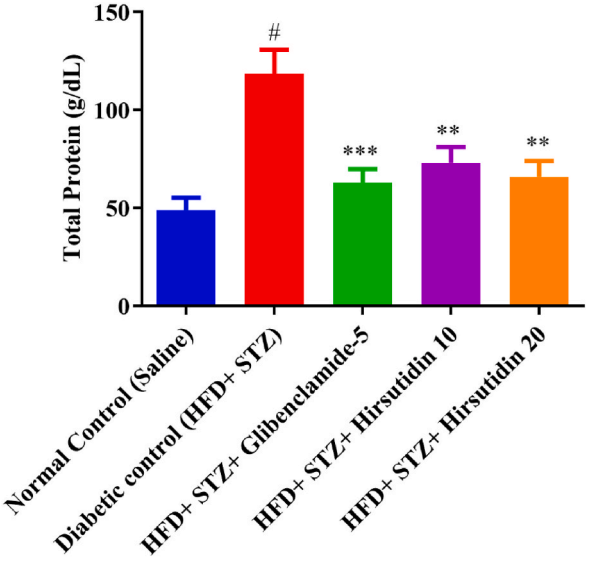

Fig. 5. Outcome of hirsutidin on total protein in HFD/STZ-induced diabetic rats. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; $^{**}P < 0.001$, $^{***}P < 0.0001$ Vs HFD + STZ.

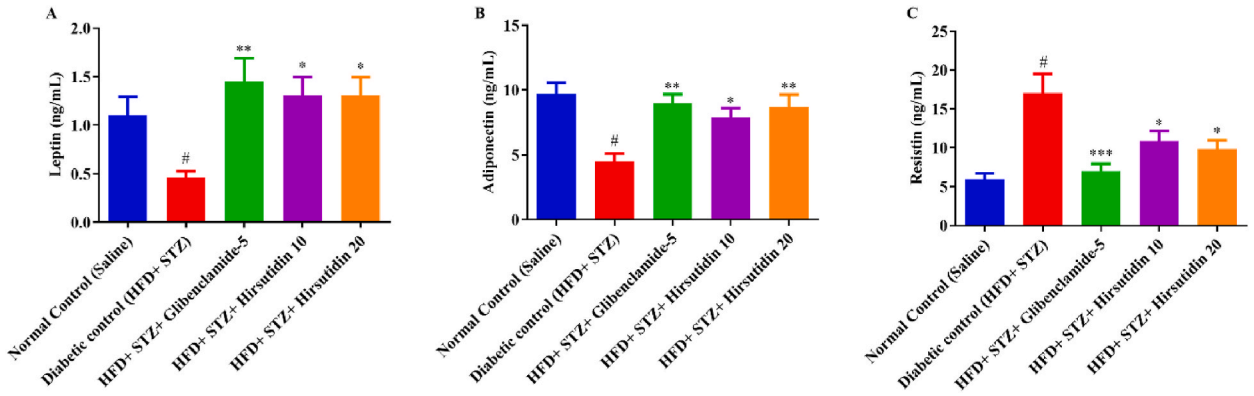

Fig. 6. A–C: Outcome of hirsutidin on leptin, adiponectin and resistin in HFD/STZ-induced diabetic rats. (A) Leptin; (B) Adiponectin; (C) Resistin. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; $^{*}P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.0001$ Vs HFD + STZ.

range. The configurations used for the previously completed dockings were used for redockings. Results obtained were subjected to make 2D and 3D images using Biovia Discovery Studio visualizer. All four structures (2AZ5, 4IBM, 6KS1, and 7Z3Q) exhibit good stereochemical quality, as evidenced by the concentration of residues in the favoured and allowed regions of the Ramachandran plots (Fig. 10A–D). Hirsutidin Proteins TNF-α (2AZ5), Insulin (4IBM), Adiponectin (6KS1), Leptin (7Z3Q) via LigPlot v1.4.5, Maestro V12.8, PLIP server and Biovia Discovery studio visualizer. Hirsutidin shows favorable binding affinities with TNF-α (2AZ5, -6.708 kcal/mol), Insulin (4IBM, -7.674 kcal/mol), Adiponectin (6KS1, -7.2 kcal/mol), and Leptin (7Z3Q, -7.547 kcal/mol) (Tables 4 and 11A-D).

4. Discussion

The antidiabetic potential of hirsutidin was explored in this work using the T2DM classical model produced by HFD/STZ. Our study results demonstrate that HFD and low doses of STZ combination could be efficaciously utilized to evaluate the antidiabetic drug [19]. *Catharanthus roseus* L. (apocyanaceae), remarkably notable as Vinca Rosea, appears to be the source of the active component hirsutidin, which has been used to cure various diseases. The current study's findings would provide preliminary experimental data on which further research emphasizing the therapeutic advantages of hirsutidin for diabetic patients with varied co-morbidities might be planned.

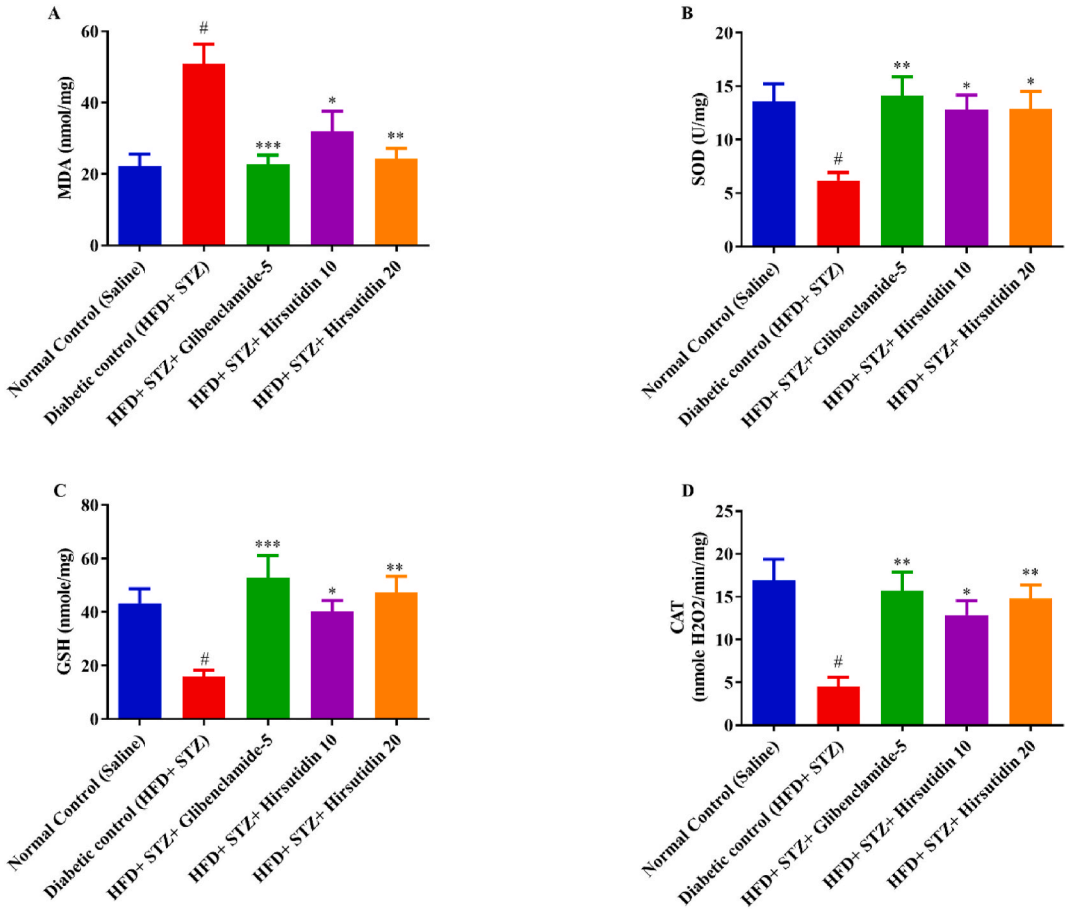

Fig. 7. A–D: Outcome of hirsutidin on oxidative and antioxidant status in HFD/STZ-induced diabetic rats. (A) MDA: malondialdehyde; (B) SOD: superoxide dismutase; (C) GSH: glutathione; (D) CAT: catalase activity. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; *P < 0.05, **P < 0.001, ***P < 0.0001 Vs HFD + STZ.

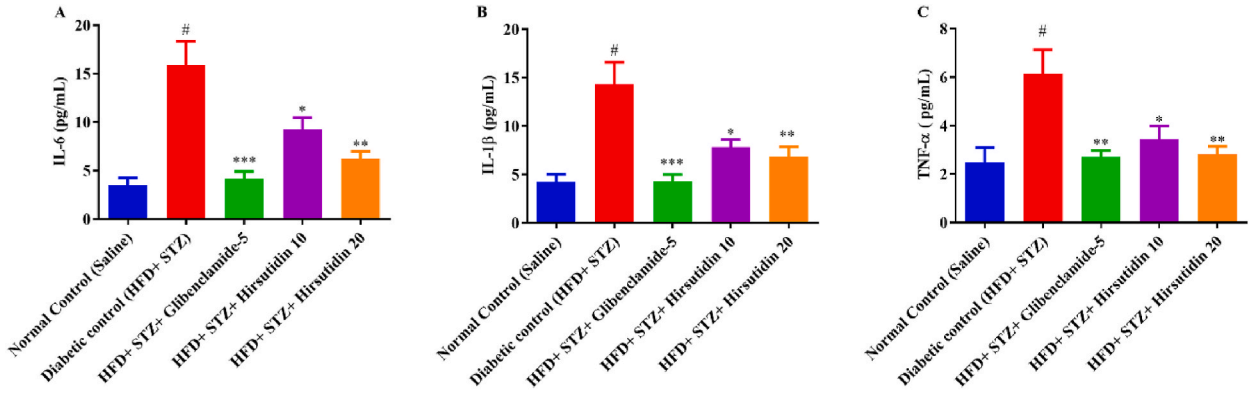

Fig. 8. A–C: Outcome of hirsutidin on proinflammatory cytokinin in HFD/STZ-induced diabetic rats. (A) Interleukin-6: IL-6; (B) Interleukin-1 β: IL-1β; (C)TNF-α: Tumor necrosis factor-α. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; *P < 0.05, **P < 0.001, ***P < 0.0001 Vs HFD + STZ.

4.1. Alter body weight

After the investigation, there was a substantial drop in the body mass in the HFD/STZ group. Furthermore, HFD/STZ group dramatically decreases body weight in all experimentally treated rats. However, HFD-fed animals have growing body weight, whereas STZ injections result in a decline in body weight in the study rats. The fundamental scientific rationale for weight defeat was thought to

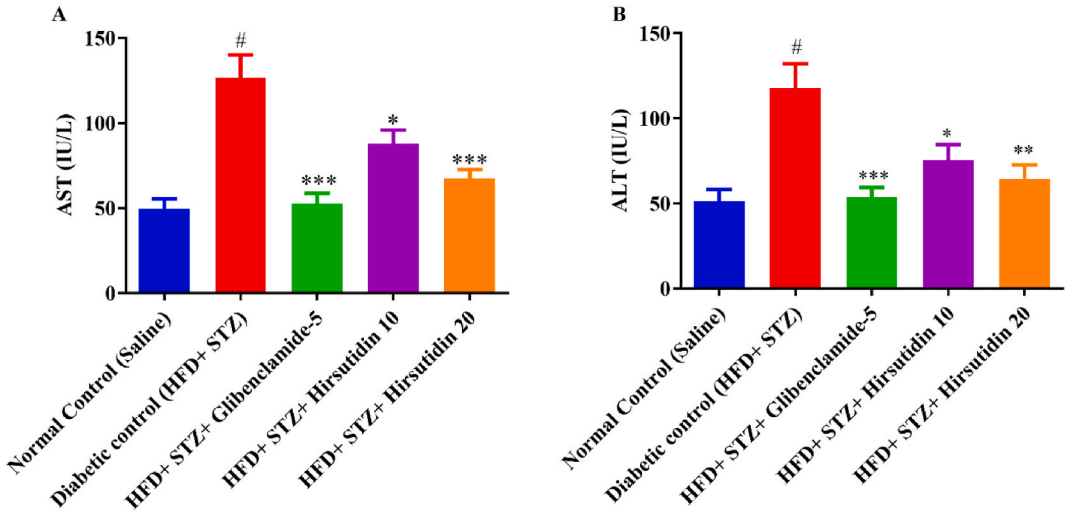

Fig. 9. A–B: Outcome of hirsutidin on liver markers in HFD/STZ-induced diabetic rats. (A) AST: aspartate aminotransferase; (B) ALT: alanine aminotransferase level. ($^{\#}P < 0.05$) Normal control Vs HFD + STZ; *P < 0.05, **P < 0.001, ***P < 0.0001 Vs HFD + STZ.

Table 3
Grid parameter.

PDB ID	CENTRE CO-ORDINATES			SIZE CO-ORDINATES		
	X	Y	Z	X	Y	Z
2AZ5	−19.41	−74.65	33.85	25	25	25
4IBM	4.31	−9.46	6.92	20	20	20
6KS1	12.596	35.981	30.117	30	30	30
7Z3Q	−36.88	−74.536	30.568	30	30	30

be insulin insufficiency or reduced sensitivity of muscle tissue to insulin, leading to protein breakdown and weight loss. Adipose mobilization of adipose tissue induced by insufficiency or insulin resistance leads to weight loss and increased lipids [36,38,39]. Our findings are consistent with earlier research [40,41].

4.1.1. Diabetes mellitus

The current study findings validated hirsutidin hypoglycemic effects in diabetic rats. Blood glucose was considerably attenuated in the hirsutidin treatment compared to the HFD/STZ cluster. The antidiabetic activity of hirsutidin is corroborated by a prior study, which found that *Catharanthus roseus* ethanolic extract of flower and leaves revealed a dose-dependent reduction in blood glucose compared to the conventional medicine, glibenclamide. The antidiabetic potential of alkaloids extracted from *Catharanthus roseus* and medicine produced from the flora were sold as a diabetic therapy under the proprietary name Vinculin [23,42]. Dysregulation of the insulin signaling system, which governs glucose transport in hepatic cells, is a fundamental predictor of the glycemic result seen in uncontrolled diabetes. In addition, the conventional medicines glibenclamide and hirsutidin as a test drug recovered reduced insulin compared to HFD/STZ-provoked diabetic animals. Insulin insufficiency or resistance, which increases fatty acid and triglyceride production in adipose tissue and the liver, might be the cause of dyslipidemia [43].

4.1.2. Dyslipidemia

Dyslipidemia is a defining feature of metabolic syndrome [39]. Hypercholesterolemia may be caused by enhanced dietetical cholesterol absorption from HFD in people with diabetes. TG and TC levels were considerably more significant, whereas HDL was significantly lower in the HFD/STZ-elicited disease animals compared to saline-treated rats. However, animal groups treated with hirsutidin revealed a notable restoration in lipid profile. The hypolipidemic efficacy of *Catharanthus roseus* leaf juice was evidenced by a decrease in TG, TC, and low and very low-density lipoprotein along with histopathological changes of the liver, kidney and aorta [44]. Decreased plasma protein content has been linked to STZ-induced diabetes. Hirsutidin treatment has appreciably normalized the content of protein. The ability of hirsutidin to achieve desired lipoprotein levels implies that the plant may be able to protect against diabetes-related comorbidities.

4.1.3. Oxidative stress and antioxidant status

A considerable body of data suggests that the oxidative stress phenomenon is essential in the exploitation of T2DM-related problems, including cardiovascular abnormalities [45–47]. When there is an abundance of pro-oxidants compared to antioxidant

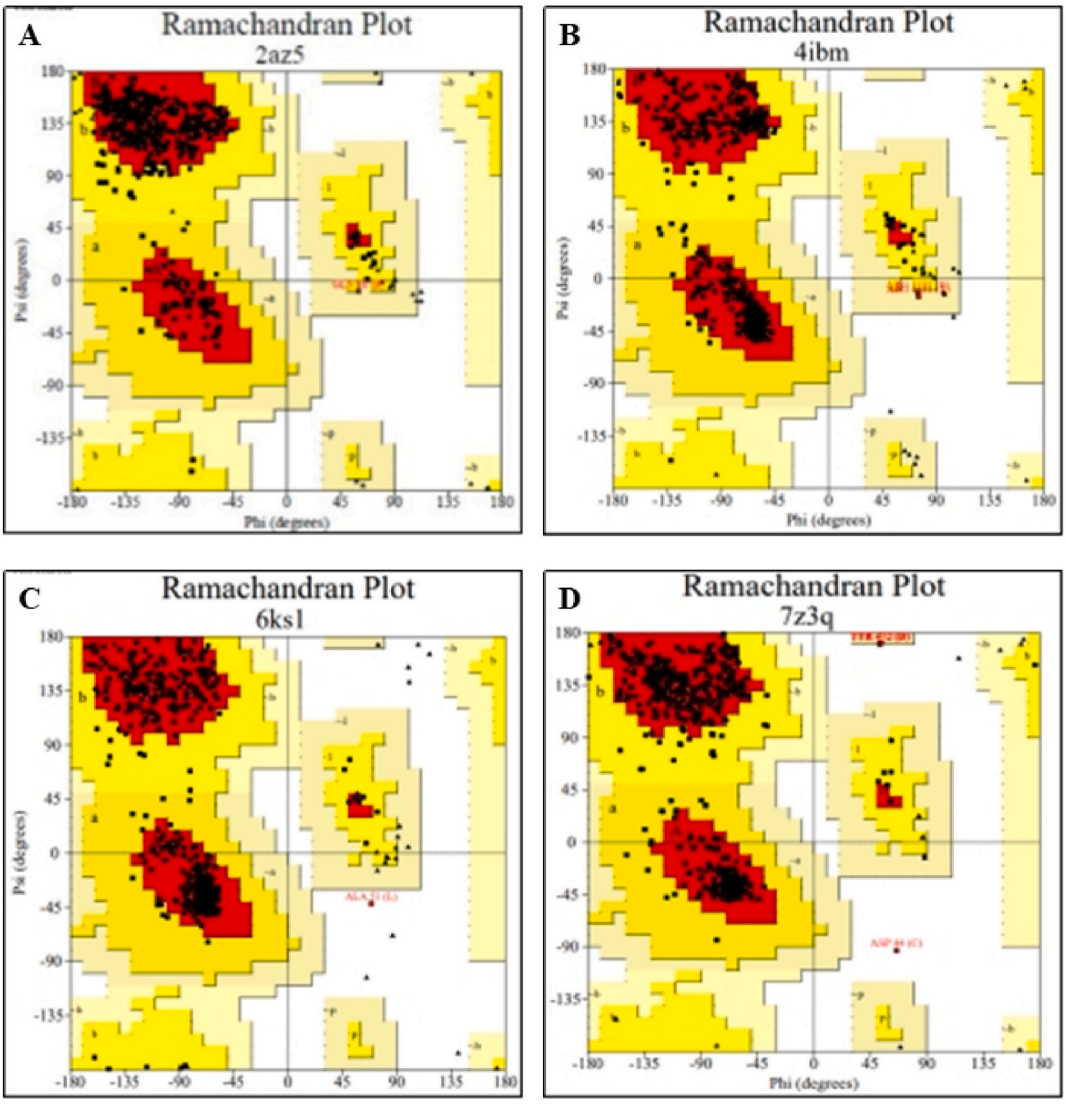

Fig. 10. A–D: Ramachandran Plot (A) 2AZ5, (B) 4IBM, (C) 6KS1 and (D) 7Z3Q obtained from PROCHECK server.

defence, oxidative stress emerges. Hyperglycemia and hyperlipidemia were linked to exaggerated reactive oxygen species (ROS) creation, inflammation and oxidative stress [48]. Liver enzymes that leak into the circulation due to lipid peroxidation impairment in the hepatic tissue, may be produced by STZ-induced T2DM in this scenario. The efficacy of hirsutidin to prevent lipid peroxidation may be due to free radical quenching enhancing the endogenous antioxidant system, allowing for the regeneration of endogenous antioxidants. *Catharanthus roseus* includes advanced flavonol glycosides and phenolic chemicals, such as caffeoylquinic acids, which have antioxidant potential. It acts as an antioxidant against ROS, which is a vital facet of the body's defence mechanism [49]. Our data was also supported by a previous study that showed hirsutidin protects against hepatic injury by alcohol-induced oxidative stress in mice [27].

4.1.4. Alter adiponectin, leptin, and resistin levels

Adiponectin is a blood plasma adipokine generated primarily on adipose tissues and has been demonstrated to immediately sensitize the body to insulin through the AdipoR1 and AdipoR2 receptors [50]. In HFD/STZ-induced rats, leptin and adiponectin levels were shown to be significantly lower [51]. In our investigation, hirsutidin supplementation, as well as regular medicine, can particularly ameliorate these adipokine and leptin level abnormalities. As a result, our findings are consistent with earlier research highlighting the function of adiponectin in boosting glucose intake and lipid oxidation [52]. Leptin is an essential hormone that inhibits appetite and helps prevent overeating [53]. Adiponectin activities need AMP-activated protein kinase activation in the hepatic and skeletal muscles and are correlated with more excellent cellular fat-burning [54].

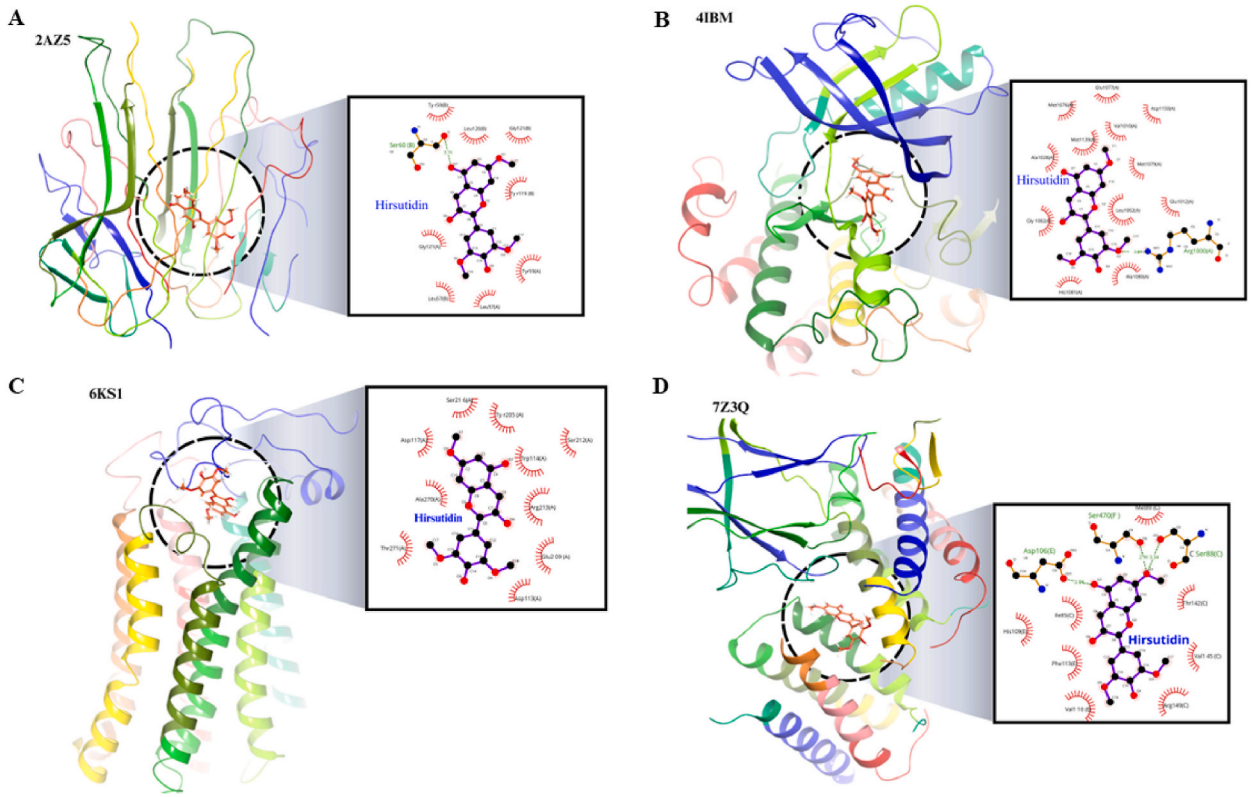

Fig. 11. A–D: Molecular docking of hirsutidin Proteins (A) TNF-α (2AZ5), (B) Insulin (4IBM), (C) Adiponectin (6KS1), and (D) Leptin (7Z3Q) Proteins.

Table 4
Docking Score and Intermolecular Interactions of ligands Hirsutidin Proteins TNF-α (2AZ5), Insulin (4IBM), Adiponectin (6KS1), Leptin (7Z3Q) using PLIP server, Maestro V12.8, LigPlot v1.4.5 and Biovia Discovery studio visualizer.

Sr. No.	Name of Compound	Binding Energy	Type of Interaction	Residue Id	Distance In A^0
1	2AZ5	−6.708	Hydrophobic Interactions	TYR59A	3.99
				TYR59B	3.92
				TYR119B	3.62
2	4IBM	−7.674	Hydrophobic Interactions	LEU1002A	3.98
				LEU1002A	3.09
				LEU1002A	3.67
				VAL1010A	3.92
			Hydrogen Bonds	ARG1000A	2.06
				ARG1000A	3.03
				MET1079A	3.2
3	6KS1	−7.2	Hydrophobic Interactions	TYR205A	3.55
				ARG213A	3.49
			Hydrogen Bonds	SER212A	2.15
				SER216A	3.03
4	7Z3Q	−7.547	Hydrophobic Interactions	ILE85C	3.77
				HIS109E	3.58
				VAL110E	3.9
				PHE113E	3.85
				PHE113E	3.64
			Hydrogen Bonds	SER88C	2.64
				ARG149C	2.76
				SER470F	2.51

4.1.5. Inflammation

Similarly, adiponectin functions as an anti-inflammatory cytokine, which is critical in the aetiology of T2DM [55]. Hirsutidin could cut down inflammation by down-regulating other inflammatory cytokines, which are supported by a previous study [23]. Resistin is a

putative adipokine from the cysteine-rich protein family that raises insulin and blood glucose levels while limiting the hypoglycaemic consequence of insulin infusion. The efficacy of hirsutidin to reduce serum resistin was demonstrated in this investigation. The test medicine's approach to achieving optimal hormonal equilibrium may make it a better complement to reduce excessive ingestion, eventually leading to obesity.

4.1.6. Molecular docking of hirsutidin

Also, molecular docking studies were undertaken to investigate critical targets associated with diabetes, including TNF-α, insulin, adiponectin and leptin. Intermolecular Interactions of ligands and docking score Hirsutidin Proteins TNF-α (2AZ5), Insulin (4IBM), Adiponectin (6KS1), Leptin (7Z3Q) using PLIP server, Maestro V12.8, LigPlot v1.4.5 and Biovia Discovery studio visualizer. Insulin (4IBM) showed favorable binding energy (-7.674 kcal/mol) as compared to other compounds. These findings revealed that hirsutidin may be a promising therapeutic agent that targets TNF-α (2AZ5), Insulin (4IBM), Adiponectin (6KS1), Leptin (7Z3Q) as critical targets for further exploration in the context of HFD/STZ diabetes-induced rat.

Hirsutidin may exert its anti-diabetic effects through multiple mechanisms, including enhancing insulin signalling by interacting with the insulin receptor, which improves insulin sensitivity and promotes glycogen synthesis, thereby reducing hyperglycemia. Additionally, hirsutidin may regulate lipid metabolism by upregulating adiponectin, leading to reduced total cholesterol and triglycerides while increasing high-density lipoprotein levels. The compound also appears to reduce oxidative stress by enhancing the activity of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione while decreasing malondialdehyde levels. Furthermore, hirsutidin likely exerts anti-inflammatory effects by inhibiting pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF-α, possibly through direct interaction with TNF-α. The reduction in liver enzymes suggests a hepatoprotective effect, potentially due to reduced oxidative stress and inflammation, along with improved lipid metabolism. Moreover, hirsutidin may modulate adipokine secretion by lowering leptin and resistin levels and increasing adiponectin, thereby improving leptin sensitivity and energy balance.

It was discovered that hirsutidin treatment might affect putative biomarkers and their associated metabolic pathways. This study was limited by the small number of animals and the short duration of the experiment. Future research is needed to confirm this mechanism, including molecular mechanisms, Western blot and a histopathological study. Clinical studies must explore the additional mechanisms and establish their clinical utility.

5. Conclusion

Diabetes induction with HFD/STZ resulted in hyperglycemia, dyslipidemia, oxidative stress, inflammation, liver damage, and aberrant blood levels of specific hormones such as adiponectin, leptin, resistin, and insulin, as evidenced by the biochemical data in this study. The underlying molecular mechanics of action are most certainly implicated, as is hirsutidin potential to exhibit anti-diabetic actions across numerous routes. The HFD/STZ diabetes-induced rat model produced a significant reversal impact on blood levels of adiponectin, leptin, and inflammatory markers. A molecular docking analysis also validated the experimental evidence.

Data availability statement

Data will be made available on request. are included in the article. Raw data supporting this study's findings are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Naif A.R. Almalki: Writing – original draft. **Fahad A. Al-Abbasi:** Writing – review & editing. **Ehssan Moglad:** Writing – review & editing. **Muhammad Afzal:** Writing – review & editing. **Salwa D. Al-Qahtani:** Writing – review & editing. **Sami I. Alzarea:** Writing – review & editing. **Faisal Imam:** Funding acquisition. **Nadeem Sayyed:** Writing – original draft. **Imran Kazmi:** Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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