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Gedunin attenuates streptozotocin–induced diabetic hepatopathy in rats

Suchismita Mazumdar, Thankamani M Marar, Jyoti M Patki✉

School of Biotechnology and Bioinformatics, D.Y. Patil Deemed to be University, Navi Mumbai 400614, India

ABSTRACT

Objective: To examine the hepatoprotective effects of gedunin in streptozotocin (STZ)-induced diabetic rats.

Methods: Rats were divided into 4 groups: control, STZ, gedunin, and STZ+gedunin. Biochemical parameters for liver function and liver histology were studied. The molecular interaction of gedunin with the liver glucose transporters GLUT2 and SGLT1 was examined using AutoDock Vina.

Results: Gedunin attenuated STZ-induced increase in the levels of aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and gamma-glutamyl transferase in the serum and liver tissue, reduced lipid peroxidation, and enhanced antioxidant activity. Histopathological studies showed considerable restoration of liver architecture in gedunin-treated diabetic rats. *In silico* studies revealed stable binding of gedunin with GLUT2 and SGLT1.

Conclusions: Gedunin exerts hepatoprotective effects in STZ-induced diabetic rats by reducing liver enzymatic activities and oxidative stress. Further studies are warranted to verify the mechanism of its hepatoprotective action.

KEYWORDS: Gedunin; GLUT2; SGLT1; Diabetic hepatopathy; Docking; Histology; Homology modelling

1. Introduction

The diabetic therapeutic landscape is radically transforming with the increasing entry of biopharmaceuticals that confer targeted action and reduce side effects. Several traditionally known medicinal plants are reported to possess bioactives with potent antidiabetic properties. The multitude of modes of action include hypoglycaemic effect through glucosidase enzyme inhibition, modulation of cellular glucose uptake, and antioxidant effect.

However, the perturbation of glucose homeostasis in diabetes

also produces damaging effects in the long term that converge onto associated organs, primarily the liver and the kidneys. Diabetic therapy is therefore complicated due to these comorbidities limiting the success of newer drug lead compounds.

The liver is central to glucose metabolism and controls its synthesis, storage, and distribution thus maintaining glucose homeostasis. High levels of fatty acids and glucose during diabetes combined with accumulation of advanced glycation end product (AGE) precursors cause excessive formation of free radicals leading to oxidative stress. Diabetic patients are always at a risk of developing liver disorders like non-alcoholic fatty liver disease (NAFLD), cirrhosis, acute liver failure, fibrosis, or glycogenic hepatopathy[1].

Damage is attributed to gluconeogenesis and glucose absorption through the transmembrane glucose transporters expressed in the hepatocytes. The glucose transporters (GLUTs) are a family of 14

Significance

The management of diabetes is complicated due to organ injury. Hepatoprotective compounds can supplement therapy and enhance efficacy. The present study shows that gedunin improved liver enzyme profile, reduced oxidative stress, and mitigated liver histological changes in streptozotocin-induced diabetic rats. The stable binding of gedunin with GLUT2 and SGLT1 observed in the *in silico* studies suggests its protective effects possibly through modulation of these glucose transporters. However, further studies are required to verify its exact mechanism of action.

✉To whom correspondence may be addressed. E-mail: jyoti.tope@dypatil.edu

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transmembrane proteins expressed in different cells, facilitating glucose transport along a concentration gradient. Glucose transporter 2 (GLUT2) that is found in the cells of the intestinal mucosa, pancreatic islets, and renal tubules plays an important role in glucose homeostasis. GLUT2 is required for glucose-stimulated insulin secretion in the pancreatic cells, glucose output in the hepatocytes, and primary dietary glucose absorption in the intestinal cells. GLUT2 pathway in the intestine involves its glucose-induced translocation to the apical side of the membrane. Sodium-glucose cotransporters (SGLTs) are another family of glucose transporters with 12 members. While SGLT 1, 2, 4, and 5 are transporters, SGLT 3 is a glucose sensor. SGLT1 is a primary mediator for glucose absorption in the small intestine and glucose reabsorption in the kidneys[2]. The transporter proteins have therefore emerged as promising therapeutic targets for antidiabetic formulation with positive effects on associated organ physiology. To this day, several plant-derived inhibitors of glucose transport have been discovered that mainly target GLUT 1-4, 5, and 7 and SGLT 1 and 2. Herbal compounds like phlorizin, quercetin, apigenin, and tiliroside have been reported to inhibit GLUT and SGLT family of glucose transporters. Therefore, the characterization of naturally occurring small molecule inhibitors of glucose transporters would supplement diabetic therapy by extending to organ protection and prevention of comorbidities[3]. In the present study, we aimed to investigate the hepatoprotective effect of gedunin on streptozotocin (STZ)-induced diabetic rats using *in silico* and *in vivo* studies.

2. Materials and methods

2.1. Drugs, chemicals and instruments

Gedunin was purchased from Santa Cruz Biotechnology, Inc. India. All other chemicals purchased were of analytical grade. The Accu-chek glucometer for blood glucose determination was purchased from Roche Diabetes Care India Pvt. Ltd. Biochemical analysis was done on Mispal Viva semi-automated analyser following manufacturer's instruction.

2.2. Animal model

Adult male Wistar rats [four weeks old and an average weight of (140±20) g] were purchased from Bharat Serum and Vaccines Ltd., Thane, India. The rats were housed in clean, sterile, polypropylene cages and acclimatized for 7 d at a standard temperature (25 ± 2) °C and humidity (70 ± 5)%, and a 12 h light and dark cycle according to guidelines laid out by the Committee of Purpose of Control and Supervision of Experiment on Animals.

2.3. Experimental protocol

After the adaptation period, four groups were made with six animals in each group. Group 1: Control group (without diabetes and drug treatment); Group 2: Diabetes control group (rats induced with diabetes without any drug treatment); Group 3: Drug control group (non-diabetic rats treated with gedunin); Group 4: Treated diabetes group (rats induced with diabetes and treated with gedunin). All animals were given normal animal feed throughout 28 d. After fasting overnight, groups 2 and 4 were administered a single dose (50 mg/kg body weight) of STZ diluted in 0.1 M Na-citrate buffer. All STZ-injected rats were given 10% sucrose in drinking water for 3 d following the injections to prevent hypoglycemia. Three days (72 h) following STZ injection, blood glucose levels were determined and rats with blood glucose levels higher than 250 mg/dL were considered diabetic. A dose of 1 mg/kg body weight of gedunin was administered through oral gavage three times a week for 2 weeks to group 4. Dosage was decided based on a pilot study. Non-diabetic rats of group 3 received the same gedunin treatment as group 4 and served as drug control. On completion of two weeks of drug treatment, rats of all the groups were sacrificed by cervical decapitation. Immediately after the sacrifice, blood and organs were collected.

2.4. Blood preparation for serological analysis

Blood was collected in vacutainers without anticoagulant coating and allowed to stand for 10 min. After clotting, tubes were centrifuged at 2000 ×g and serum was obtained by aspiration. The levels of liver marker enzymes namely, aspartate transaminase (AST)[4], alanine transaminase (ALT)[4], alkaline phosphatase (ALP)[5], lactate dehydrogenase (LDH)[6] and gamma-glutamyl transferase (GGT)[7] in serum were determined according to manufacturer's protocol (ACCUREX, Biomedical Pvt. Ltd). All serological assays were performed within 12 hours of serum preparation.

2.5. Tissue preparation for biochemical and histological analysis

The liver was immediately dissected out and washed in ice-cold normal saline. For biochemical analysis, liver tissues were accurately weighed and homogenized with the help of a glass Teflon homogenizer in 0.01 M Tris-HCl buffer (pH 7.4) for enzyme assays and 0.15 M KCl for lipid peroxidation study. The aliquots of homogenate were suitably processed for the assessment of biochemical parameters. The levels of liver antioxidants like peroxides (LPx)[8], catalase[9], superoxide dismutase (SOD)[10], glutathione reductase (GRD)[11], glutathione (GSH)[12] and liver marker enzymes including AST, ALT, ALP and LDH were assayed. For histopathological study, the liver tissue samples were fixed in

4% formalin for 2 h. Three short-axis slices of tissues were obtained. The tissues were embedded in paraffin blocks. With the help of a semiautomated microtome, MRM-ST, SAFIRE, sectioning was done to obtain sections of 3–4 μm thickness. Xylene was used to deparaffinize the sections and haematoxylin and eosin (H&E) were used for staining. Stained liver sections were microscopically examined (400 $\times$ magnification) using a light microscope equipped with a digital camera (Olympus CX23 HD digital). Photomicrographs of the tissue sections were captured.

2.6. Homology modeling of GLUT2 and SGLT1 protein

The homology modeling was performed in Swiss Model workspace based on the homologous template (PDB ID: 4zw9) for GLUT2 which is the crystal structure of human GLUT3 bound to *D*-glucose and PDB ID 2xq2 and 3dh4 for SGLT1. The primary sequences of human GLUT2 protein (with Uniport Sequence Accession Number: P11168) and SGLT1 (NCBI Sequence accession no.NP_000334) were obtained in Fasta format from SWISS-PROT public domain database. BLASTp was performed against Protein Data Bank (PDB) (<http://www.rcsb.org/>) to select a proper template for comparative modeling.

2.7. Structure optimization and analysis of the predicted models

All the predicted models for GLUT2 and SGLT1 were subjected to structure optimization and validation using various tools like YASARA[13], 3DRefine[14], and RAMPAGE[15] before performing docking and simulation studies. The quality of the predicted models was analyzed with SAVES (<https://www.doe-mbi.ucla.edu/saves/>) and MolProbity[16]. The modeled structure generated was validated with the Ramachandran plot analysis by using PROCHECK[17].

2.8. Molecular docking

AutoDock tools 4.2 was used for preparing the grid parameters for protein and ligands. The docking was performed by using AutoDock Vina[18].

The optimized GLUT2 protein was subjected to molecular docking with gedunin, quercetin, and glucose while optimized SGLT1 was subjected to molecular docking with gedunin, mizagliflozin, and glucose. The CIDs for gedunin, quercetin, *D*-glucose and mizagliflozin retrieved from PubChem database were 12004512, 5280343, 79025, and 10460535, respectively.

The 3D grid box for GLUT2 was prepared with a size of 46 $\times$ 68 $\times$ 40 grid points with a spacing of 1.0 $\text{\AA}$ at the geometric center of the target enzyme. The grid center was kept as 56.639, 8.779, and 59.913 for *x*, *y*, and *z*, respectively. The CID that was retrieved from

the PubChem database was 12004512 and 10460535, respectively. For SGLT1 docking, grid box dimensions were set as 22 $\times$ 28 $\times$ 22 with a grid spacing of 1.0 $\text{\AA}$ which is suitable for performing in the Vina program. The grid center was kept as 4.093, –36.400, and –51.160 for *x*, *y*, and *z*, respectively.

2.9. Statistical analysis

The results are expressed as mean $\pm$ standard deviation (SD) ($n=6$). Statistical comparison between different groups was performed by one-way ANOVA followed by Tukey's test and $P<0.05$ was regarded as statistically significant

2.10. Ethical statement

Animal studies were initiated after obtaining approval of the experimental protocol (date of approval: 03/11/2018) from the Institutional Animal Ethics Committee (IAEC-Registration number 1553/PO/a/11/CPCSEA)

3. Results

3.1. Effect of gedunin on liver marker enzymes in liver tissue and serum

Liver enzymes are indicators of liver function and health. The STZ-induced diabetic group was characterized by very high levels of liver marker enzymes in serum as well as liver tissue as compared to the control group ($P<0.01$) (Tables 1 and 2). The diabetic condition was alleviated by treatment with gedunin, as evidenced by restoration of liver enzyme activity ($P<0.01$). This trend was also found in serum. AST, ALT, ALP, LDH, and GGT levels were normalized following treatment with gedunin ($P<0.01$). The non-diabetic drug control group exhibited no significant changes compared to the control.

Table 1. Effect of gedunin on enzymatic activities in the liver tissue of streptozotocin-induced diabetic rats.

Parameter	Normal control	Diabetes control	Diabetes + Gedunin
AST	117.74 $\pm$ 13.15	242.34 $\pm$ 18.50 [#]	124.03 $\pm$ 7.27 [*]
ALT	740.37 $\pm$ 10.01	1237.00 $\pm$ 17.04 [#]	749.43 $\pm$ 14.40 [*]
ALP	125.52 $\pm$ 6.38	330.84 $\pm$ 10.43 [#]	135.17 $\pm$ 5.52 [*]
LDH	0.18 $\pm$ 0.13	0.61 $\pm$ 0.27 [#]	0.19 $\pm$ 0.03 [*]

Values are expressed as mean $\pm$ SD ($n=6$) and analyzed by one-way ANOVA followed by Tukey's test. [#] $P<0.01$ compared with the normal control group, ^{*} $P<0.01$ compared with the diabetes control group. For AST, ALT and LDH, the unit is nmoles of pyruvate/min/mg protein. For ALP, the unit is nmoles of phosphorus/min/mg protein. AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase.

Table 2. Effect of gedunin on enzymatic activities in the serum of streptozotocin-induced diabetic rats (U/L).

Parameter	Normal control	Diabetes control	Diabetes + Gedunin
AST	55.15±10.97	133.13±14.7 [#]	64.10±10.46 [*]
ALT	75.36±3.21	135.77±10.18 [#]	80.21±2.23 [*]
ALP	221.05±8.08	415.04±8.55 [#]	240.34±9.61 [*]
LDH	279.34±5.02	457.52±11.87 [#]	286.14±5.10 [*]
GGT	0.03±0.01	2.11±0.04 [#]	0.05±0.02 [*]

Values are expressed as mean ± SD ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test. [#] $P < 0.01$ compared with the normal control group, ^{*} $P < 0.01$ compared with the diabetes control group. GGT: gamma glutamyl transferase.

3.2. Effect of gedunin on antioxidant markers in the liver of STZ-induced diabetic rats

The results of oxidative status of liver tissue in control and diabetic rats are presented in Table 3. Elevated lipid peroxidation (2.94 ± 0.18 , $P < 0.01$) accompanied by declined activities of catalase, GRD, SOD, and GSH was observed in STZ-induced diabetic rats. A significant improvement in all indices was observed in rats receiving 1 mg/kg body weight gedunin ($P < 0.01$). The group treated with gedunin alone showed no significant changes in oxidative status compared to the control.

3.3. The therapeutic effect of gedunin against STZ-induced liver histopathology in rats

The histological changes in liver tissue were evaluated using

hematoxylin and eosin staining. The normal architecture of normal control animals was characterized by large polygonal hepatic cells, radiating out from the central vein with eosinophilic cytoplasm and distinct round nuclei. Hepatic cords were separated by sinusoids with stellate Kupffer cells (Figure 1A). In rats with STZ-induced diabetes, distinct signs of liver injury were observed including hepatocyte necrosis, steatosis, and lobular and periportal inflammation. Hepatocellular ballooning and degeneration led to the disruption of the normal hepatic cord structure that now appeared like hollow vacuoles (Figure 1B). Gedunin treatment improved liver histology by partially reversing tissue necrosis and inflammatory cell infiltration (Figure 1D). Liver morphology remained undisturbed and resembled that of the normal control in rats treated with gedunin alone (Figure 1C).

3.4. Structure optimization and molecular docking

SWISS-MODEL and BLAST showed that the template for GLUT2 (PDB ID: 4zw9) had 53.59% sequence identity to the query sequence (Uniport Sequence Accession Number: P11168) with 1.50 Å resolution, while the template for SGLT1 (PDB ID 2xq2 and 3dh4) had 30.59% sequence identity to the query sequence (NCBI Sequence accession no.NP_000334) with 2.7 Å resolution. The QMEAN score and GMQE were found to be 3.70 and 0.73, respectively (GLUT2) and -5.32 and 0.54 , respectively (SGLT1). The Ramchandran plot analysis of the modeled protein

Table 3. Effect of gedunin on biochemical parameters in liver tissue of streptozotocin-induced diabetic rats.

Parameter	Normal control	Diabetes control	Diabetes + Gedunin
LPx (μmoles of MDA/mg protein)	0.55±0.16	2.94±0.18 [#]	0.60±0.03 [*]
Catalase (μmol of H ₂ O ₂ consumed/min/mg protein)	1.08±0.19	0.36±0.14 [#]	0.93±0.12 [*]
SOD (Units/mg protein)	13.30±1.89	4.88±0.34 [#]	10.22±2.99 [*]
GSH (nmoles of GSH/mg protein)	760.19±0.17	503.60±0.04 [#]	732.21±0.14 [*]
GRD (Units/mg protein)	0.54±0.04	0.30±0.04 [#]	0.52±0.02 [*]

Values are expressed as mean ± SD ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's test.

[#] $P < 0.01$ compared with the normal control group, ^{*} $P < 0.01$ compared with the diabetes control group. LPx: lipid hydroperoxide; SOD: superoxide dismutase; GSH: glutathione; GRD: glutathione reductase.

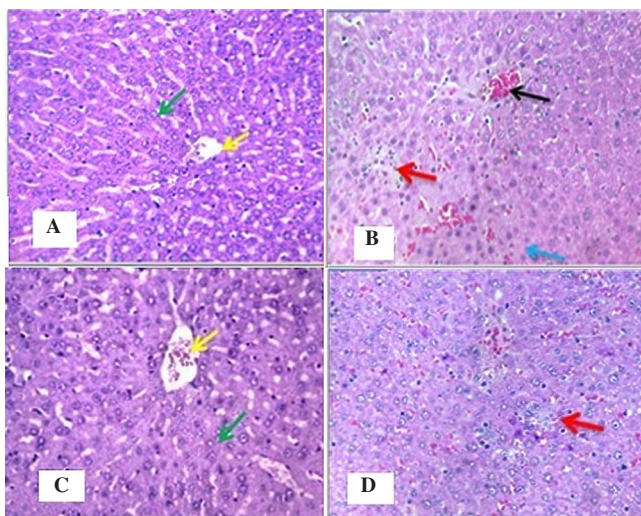


Figure 1. Haematoxylin and eosin (H&E) stained liver sections at 400× magnification. (A) The normal control shows intact hepatocytes (green arrow) radiating out from central vein (yellow arrow). (B) Diabetes control shows necrosis, vascular and cellular degeneration (red arrow), vascular congestion (black arrow) and hyperplasia of the hepatocytes (blue arrow). (C) The group treated with gedunin alone shows intact hepatocytes (green arrow) and central vein (yellow arrow). (D) The diabetes group treated with gedunin shows improved liver structure with some remaining vascular and cellular degeneration (red arrow).

using PROCHECK revealed 94.3% residues for GLUT2 and 90.3% residues for SGLT1 in the most favored region based on the PDBsum result and no amino acid residue was present in the disallowed region (Supplementary Figure).

The results of the docking study are presented in Tables 4 and 5 which document the binding free energy and the key interacting residues for each of the ligands with GLUT2 and SGLT1, respectively. The binding energies for interaction of gedunin with both the receptors alone and in the presence of the substrate (glucose) can be compared with the binding energies of the interaction of standard inhibitors (quercetin and mizaglifozin) with both receptors. The molecular interactions involve H bonding as

well as hydrophobic interactions (pi-alkyl, alkyl, pi-pi, pi-sigma, pi-cation). Many interacting amino acids were more favorable towards hydrogen bonding. The docked poses of gedunin at the binding sites of GLUT2 and SGLT1 in 3D can be observed in Figure 2 (B and D) and Figure 3 (B and D). The bound confirmations were compared with those of standard inhibitors for both the target receptors.

4. Discussion

The liver plays a crucial role in glucose homeostasis involving glycolysis, gluconeogenesis, and glycogen synthesis. Metabolic

Table 4. Molecular interactions of ligands in the binding site of GLUT2 protein.

Ligand	H-bond	Pi-alkyl	Pi-pi	Pi-sigma	Binding energy (kcal/mol)
GLUT2 + Glucose	Thr 327, Gln 204, Gln 191, Trp 442	-	-	-	-7.6
GLUT2 + Gedunin	His 190, Thr 327, Gln 204	Ala 451, Ala1 28	-	Tyr324, Ala105	-7.2
GLUT2 + Quercetin	Asn 447, Ala 451	Ala1 28, Val 101	Tyr324, Tyr325	-	-6.2
GLUT2 + Glucose + Gedunin	Thr 327, Trp 442, Val 195	Tyr 324, Gln 191	-	-	-8.5
GLUT2 + Glucose + Quercetin	Tyr 325, Asn 320	Ala 451, Val 101, I28	Tyr324	-	-6.0

Table 5. Molecular interactions of ligands in the binding site of SGLT1 protein.

Ligand	H-bond	Pi-alkyl/alkyl	Pi-pi	Pi-sigma	Pi-cation	Binding energy (kcal/mol)
SGLT1 + Glucose	Lys 157, Asp 161, His 83	-	-	-	-	-7.4
SGLT1 + Gedunin	Lys 157	Ile 297, Ile 397	His 83	-	-	-8.0
SGLT1 + Mizaglifozin	Asp 294, Ser 393, Ile 79, Ala 76	Ile 397	-	Tyr 290	-	-9.0
SGLT1 + Glucose + Gedunin	Ser 392, Ser 393	Ile 397, Ile 297, Ala199, Phe 58, Phe 59	-	-	Lys157	-8.4
SGLT1 + Glucose + Mizaglifozin	His 83, Ser 77, Tyr 290, Asp 294, Tyr 153, Ser 400	Ile 397	-	-	-	-7.8

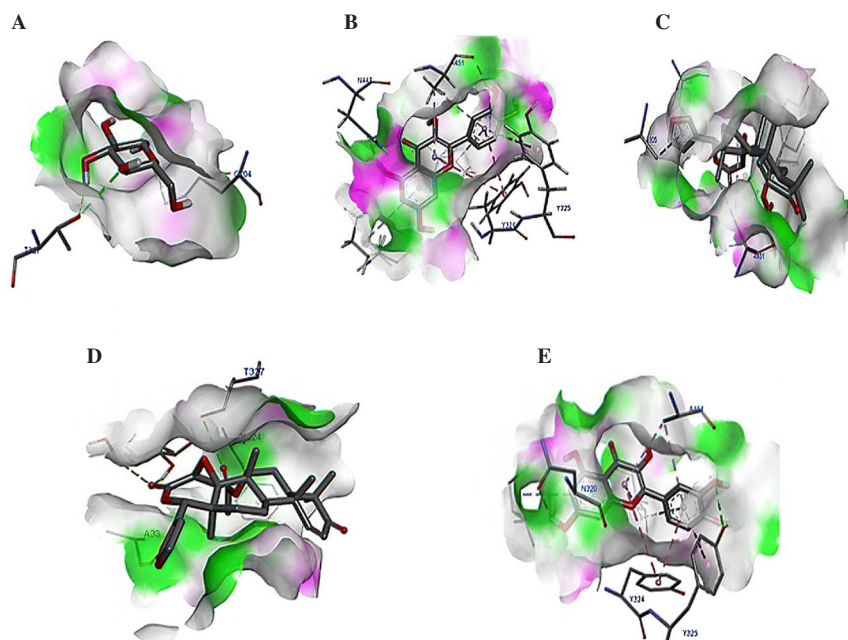


Figure 2. Molecular docking interaction pattern of ligands with the active site of GLUT2 generated using AutoDock Tools 4.2 platform. (A): 3D molecular surface view of GLUT2 docked with glucose; (B): 3D structure of GLUT2 docked with gedunin; (C): 3D structure of GLUT2 docked with quercetin; (D): 3D structure of GLUT2_Glucose complex docked with gedunin; (E): 3D structure of GLUT2_Glucose complex docked with quercetin.

defects associated with diabetes often lead to disruption of liver control mechanisms. This is attributed to insulin resistance, oxidative stress, and inflammation. While anti-diabetic therapy is focused on achieving hypoglycaemic effects, the burden on the liver remains uncorrected[19].

Extracts of plant parts are rich in constituents like phenols, coumarins, lignans, essential oils, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids, xanthenes, *etc.*, and are reported to possess hepatoprotective properties[20].

Liver function and integrity are often evaluated through the levels and activities of liver enzymes and their elevations are typical of diabetes. Considering the impact on the liver, even the severity of the diabetic condition can be predicted by liver enzyme profiles. Oxidative stress-related hepatocellular damage leads to the introduction of liver enzymes into circulation[21]. In the present study, the levels of ALT, AST, ALP, LDH, and GGT in serum were significantly elevated, possibly due to enzyme leakage from hepatocellular cytosol into blood. The high levels of these enzymes in the liver tissue on the other hand can be related to metabolic alterations due to impaired insulin restoration. Excess AST in the blood of diabetic rats was an attestation to liver/pancreatic damage and exacerbated muscle protein breakdown. ALP, as a leading preclinical biomarker for hepatobiliary or cholestatic liver injury, is frequently used to affirm the integrity of plasma membrane and endoplasmic reticulum of hepatocytes. GGT, on the other hand, is a more sensitive marker of bile duct obstruction or intrahepatic

cholestasis and oxidative stress[22,23]. GGT was significantly increased in diabetic conditions when all the antioxidants in the study were decreased. High serum transaminases are indicative of increased insulin resistance and defective glucose utilization by the liver[24]. Hepatotoxicity is a well-documented effect of STZ[22] and the observed rise in the liver enzymes in diabetic animals, points towards an adversely affected liver.

Unperturbed activities of hepatic enzymes in gedunin-treated normal rats can be considered as data affirming the absence of any adverse effects of gedunin. According to the near-control levels of almost all liver enzymes, it can be assumed that gedunin prevented excessive liver damage and maintained tissue integrity in even adverse metabolic conditions. Previous studies have reported similar beneficial effects of phytochemicals in preventing and even reversing hepatic damage[20,25,26].

Considering hyperglycemia, generation of oxidative stress, and inflammation as the major drivers of organ deterioration in diabetes, gedunin and similar compounds must play a hypoglycaemic, antioxidant, and/or anti-inflammatory role to manifest their therapeutic benefits. Previous studies have reported that gedunin exhibits hypoglycemic effect through the inhibition of amylase enzyme[27]. Gedunin has also been credited with possessing anti-inflammatory effects in a study reported by Braga *et al.*[28]. *Azadirachta indica*, the plant source of gedunin, used in the present study is listed in the monographs of WHO among medicinal plants possessing anti-diabetic potential[25].

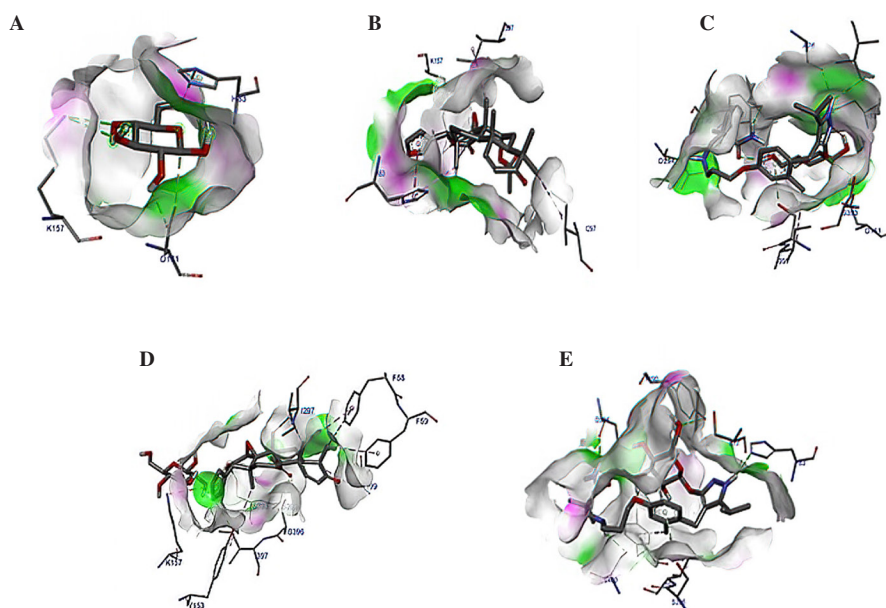


Figure 3. Molecular docking interaction pattern of ligands with the active site of SGLT1 generated using AutoDock Tools 4.2 platform. (A): 3D molecular surface view of SGLT1 docked with glucose; (B): 3D molecular surface view of SGLT1 docked with gedunin, (C): 3D molecular surface view of SGLT1 docked with mizagliflozin; (D): 3D molecular surface view of SGLT1_Glucose complex docked with gedunin; (E): 3D molecular surface view of SGLT1_Glucose complex docked with mizagliflozin.

Oxidative stress is a condition that emerges from an imbalance between the formation and neutralization of reactive oxygen species (ROS). Enhanced ROS production and decreased scavenging activity result in extensive tissue damage. Oxidative stress in diabetes is attributed to excessive oxygen radical formation and antioxidant enzyme glycation restricting their ability to detoxify free radicals[29]. Chronic hyperglycaemia is majorly responsible for the induction of oxidative stress through glucose auto-oxidation, polyol pathway, formation of advanced glycation end products (AGE), and PKC β 1/2 kinase activation. Increased circulating free fatty acids, leptin, and factors may also contribute to overproduction of ROS[30] and damage to cell membranes[31]. Hepatocytes are equipped with antioxidant enzymes such as catalase, SOD, glutathione peroxidase, and glutathione transferase. Early-stage diabetes generally presents an increase in SOD and catalase in response to elevated ROS production. However, long-term and progressive production exhausts the stimulation of enzymatic activity[1]. Severely depleted activity of SOD, catalase, and GRD in the liver tissues of diabetic rats is an indication of intense oxidative stress and liver damage. The elevated level of lipid peroxidation further affirmed the presence of high oxidative stress. The reversal of these changes in gedunin-treated diabetic rats is attributed to its antioxidant potential. Chloroform extracts of *Azadirachta indica* leaves are reported to cause an attenuation of non-enzymatic glycation, inhibited AGE formation, increased activity of antioxidant enzymes, and reduced lipid peroxidation[25]. Ethanol extract of *Justicia spicigera* provided protective effects against oxidative liver damage in STZ-induced diabetic rats when administered orally for 30 d majorly because of its antioxidant properties[31]. In light of the crucial role of oxidative stress in liver diseases, antioxidants are understandably considered as a good therapeutic strategy for the treatment of liver disorders.

In the present study, the histopathological changes observed in STZ-induced diabetic rats confirm the hepatotoxic effects of STZ. The loss of normal architecture in diabetes mellitus is often attributed to oxidative stress, inflammatory changes with inflammatory cell infiltration, lipid deposition, Kupffer cell hyperplasia, and perturbation of signaling mechanisms[1,22]. Therefore, an improvement in these histological changes in gedunin-treated diabetic rats indicates multifaceted potential of this phytochemical. Ethanolic root extracts of *Ageratum conyzoides* were shown to possess hepatoprotective properties and a preliminary phytochemical analysis showed the presence of terpenoids along with other compounds[32]. Aqueous extract of *Azadirachta indica* in STZ-induced diabetic rats reversed the deteriorative effects of diabetes on the liver and other essential organs[33]. Treatment with the aqueous extract of *Actinidia deliciosa* or kiwifruit improved liver function biomarkers, antioxidant status, and liver architecture in rats with STZ-induced oxidative stress[34]. In another study,

spinach methanolic extract demonstrated hepatoprotective effects through antioxidant and anti-inflammatory properties[35]. Minimal inflammatory cell infiltrates and more apparent intact hepatocytes were observed in aliskiren-treated STZ-induced diabetic rats compared to the periportal and perivascular inflammatory cell infiltrates and degenerative changes of hepatocytes of untreated groups[24]. Among other mechanisms of restoring liver architecture by gedunin, the GLUT2/ SGLT1 modulation should be taken into consideration especially based on previous reports of investigations on the role of SGLT and GLUT inhibitors in rectifying the hepatic histological damages in patients with hyperglycaemia-induced non-alcoholic steatohepatitis[36].

Crucial to the carbohydrate handling are the glucose transporter molecules GLUT and the sodium and glucose co-transporters, SGLT. GLUT is known to be involved in tissue-specific glucose uptake but hepatic GLUT2 has a major role in glucose sensing, hepatic glucose output, and regulating glucose efflux[37]. SGLTs are a group of six isoforms, of which SGLT1 and SGLT2 are known to cause glucose uptake majorly in the intestinal and renal cells, respectively[2], but are also reported to be expressed in the liver[38]. Recent literature suggests the therapeutic benefits of targeting these transporter molecules in metabolic conditions whose expressions are altered in diseased states[2].

The *in silico* docking studies involving GLUT2 showed that both the test lead molecule gedunin and substrate glucose showed almost the same binding affinity with the target protein and the binding of gedunin was more promising than the standard inhibitor quercetin. Many active site residues like Trp 442, Gln 191, His 190, and Val 195 participating in bonding between the GLUT2 and gedunin, GLUT2 and glucose, and also between the GLUT2-glucose complex docked with gedunin are common. The participation of these amino acids between GLUT2 and other ligands was also mentioned in a few earlier studies. According to those studies, Trp 442 is an important amino acid responsible for the transportation of glucose[39,40]. The involvement of Trp 442 in bond formation in the GLUT2-glucose-gedunin ternary complex strongly suggests a high probability of glucose transport modulation by gedunin. The increased binding affinity (as predicted by docking score) of gedunin to GLUT2 in the presence of its substrate glucose is another interesting finding. It can be proposed that the presence of substrate is increasing the specificity of gedunin towards the glucose transporter. However, a similar positive influence with respect to specificity of the substrate was not observed for quercetin binding. It was further noticed that gedunin binds to glucose binding sites with H-bonds, which was evident from their common amino acid residues (Thr 327, Gln 204).

In this study, it is clear that quercetin was not binding at glucose binding site, and this finding was in consonant with the findings of previously available literature where it had been documented

as a non-competitive inhibitor of GLUT2[20]. On the contrary, the binding pattern of gedunin indicates this compound to be a probable competitive inhibitor that competes with glucose for its binding to GLUT2 thereby halting the association of glucose to GLUT2 for transportation, which is ideal in postprandial conditions.

Many previous studies documented that SGLT2 inhibition significantly reduces the concentration of these enzymes and improves hepatic functions[41,42]. Phlorizin, the dual inhibitor of SGLT1/SGLT2, has been documented to improve non-alcoholic fatty liver in diabetic mice.

In the case of SGLT1, more favorable and stronger binding of gedunin to the SGLT1-Glucose binary complex was observed compared to the standard mizagliflozin, with binding energy as low as -8.4 kcal/mol. Binding with SGLT1 alone, however, was stronger for mizagliflozin compared to both glucose (substrate) as well as gedunin with the lowest binding energy of -9.0 kcal/mol. As shown in the images of docked complexes and the list of bonding amino acid residues, it was evident that gedunin binds to a site distinct but close to the mizagliflozin binding site. The amino acid residue Ile 397 was found to be shared in the binding sites of all the four complexes, namely, gedunin-SGLT1, mizagliflozin-SGLT1, gedunin-binary complex SGLT1-glucose, and mizagliflozin-binary complex SGLT1-glucose. Few residues present in the binding site of SGLT1/2 are well documented in previous literature where mizagliflozin, trilobatin, and phloridzin were used as SGLT1/2 inhibitors[43,44]. However, the residues involved in the binding site of gedunin have not been previously documented. Based on these findings, a distinct binding site of gedunin in SGLT1 protein is suggested.

However, further MD simulation analysis will be required to validate the docking results and to determine the stability and structural constancy of the complexes studied here.

In the present study, the effect of gedunin, a plant-derived bioactive compound, on the liver physiology in diabetes was examined. This study involves analysis of liver marker enzymes, antioxidant enzymes, and liver histology to assess the hepatoprotective effects of gedunin under diabetic conditions. The docking studies for the determination of molecular interaction of gedunin with glucose transporters shed light on the possibility of modulation of these transporters by gedunin. This study speculates that the molecular interaction may serve as one of the mechanisms by which gedunin produces its hepatoprotective effects. However, further analysis will be required to substantiate this relation.

To summarize, gedunin alleviated STZ-induced hepatic damage evidenced by improved liver enzymatic activities and histological changes. These findings certainly warrant further studies to probe into the exact mode of action of hepatoprotective effects of gedunin.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

TMM was involved in conception and design of the study, experimentation, data analysis and interpretation and critical review of the manuscript. JMP contributed to study design, experimentation, data analysis and interpretation, writing and review of the manuscript. SM performed the docking studies and the *in vivo* experiments, data analysis and interpretation, wrote the computational study sections of the manuscript and involved in writing other sections. All authors reviewed and approved the manuscript

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