IN SILICO EVALUATION OF THE ANTIDIABETIC ACTIVITY OF THE C-4 COMPOSITION

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Abstract. *Diabetes mellitus is a widespread endocrine-metabolic disorder characterized by persistent hyperglycemia, and the search for new effective and safe antidiabetic agents remains an urgent task. The present study was carried out to evaluate, using in silico molecular docking analysis, the possible antidiabetic mechanisms of the main components of the C-4 composition — L-carnitine, ammonium glycyrrhizinate, quercetin, and cysteine — which were investigated in an alloxan-induced experimental diabetes model. Two target proteins directly involved in antihyperglycemic mechanisms were selected: alpha-glucosidase, represented by human maltase-glucoamylase (PDB ID: 2QMJ), and dipeptidyl peptidase-IV (DPP-4, PDB ID: 1X70). Receptor and ligand structures were prepared using PyMOL and AutoDock Tools, and docking calculations were performed with AutoDock 4.2; the results were evaluated on the basis of binding free energy and the non-covalent interactions formed with active-site amino acid residues. Among the studied ligands, ammonium glycyrrhizinate showed the strongest binding to both targets, with binding energies of −10.2 kcal/mol for alpha-glucosidase and −13.2 kcal/mol for DPP-4, followed by quercetin (−8.4 and −7.6 kcal/mol, respectively). L-carnitine (−4.7 and −4.8 kcal/mol) and cysteine (−4.0 and −4.1 kcal/mol) exhibited weaker binding. These findings suggest that the antidiabetic potential of the C-4 composition may be mediated through several complementary mechanisms, including the inhibition of intestinal carbohydrate hydrolysis and the support of incretin-mediated insulin secretion, with ammonium glycyrrhizinate and quercetin acting as the main contributing components. The in silico results provide additional molecular evidence supporting the hypoglycemic effect observed under experimental conditions, while further experimental studies are required to confirm these interactions.*

Keywords: *diabetes mellitus, alloxan-induced diabetes, C-4 composition, molecular docking, in silico analysis, alpha-glucosidase, DPP-4, ammonium glycyrrhizinate, quercetin, L-carnitine, cysteine, antidiabetic activity.*

Introduction: Diabetes mellitus is one of the most widespread endocrine-metabolic disorders, characterized mainly by persistent elevation of blood glucose levels, known as hyperglycemia. This pathological condition is associated with disturbances in carbohydrate, lipid, and protein metabolism and may lead to the development of various complications affecting the cardiovascular system, kidneys, liver, and nervous system. Therefore, the search for new effective and safe agents aimed at controlling blood glucose levels remains an urgent task in the treatment and prevention of diabetes mellitus. In experimental pharmacology, alloxan is widely used to

induce experimental diabetes mellitus. Alloxan exerts a toxic effect on pancreatic β-cells, leading to decreased insulin secretion and the development of hyperglycemia. For this reason, the alloxan-induced diabetes model is considered an important experimental model for evaluating the hypoglycemic activity of new antidiabetic compounds.

The development of hyperglycemia in diabetes mellitus is not only related to insulin deficiency but also to intestinal carbohydrate digestion and glucose absorption into the bloodstream. In this process, alpha-glucosidase plays an important role, as it participates in the breakdown of complex carbohydrates into glucose. Inhibition of this enzyme can slow glucose absorption and help reduce postprandial hyperglycemia. In addition, the incretin system plays a significant role in the regulation of blood glucose levels. Dipeptidyl peptidase-IV (DPP-4) rapidly degrades incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Inhibition of DPP-4 may prolong the biological activity of GLP-1, support glucose-dependent insulin secretion, and contribute to the reduction of blood glucose levels. In recent years, in silico approaches, particularly molecular docking analysis, have been widely used to support experimental findings at the molecular level. Molecular docking makes it possible to evaluate the binding energy between a ligand and a target protein, the orientation of the ligand within the active site, and its interactions with amino acid residues. This approach is important for explaining the possible mechanism of action of biologically active compounds. In the present study, molecular docking analysis was performed to evaluate the possible molecular mechanisms underlying the antidiabetic activity of the main components of the C-4 composition, including L-carnitine, ammonium glycyrrhizinate, quercetin, and cysteine, which were studied in an alloxan-induced experimental diabetes model. For this purpose, two protein targets involved in antihyperglycemic mechanisms were selected: alpha-glucosidase, represented by human maltase-glucoamylase (PDB ID: 2QMJ), and dipeptidyl peptidase-IV, DPP-4 (PDB ID: 1X70). The formation of hyperglycemia in diabetes mellitus is a complex pathophysiological process and is not limited only to reduced insulin secretion or impaired insulin action in peripheral tissues. Intestinal enzymatic degradation of carbohydrates, glucose absorption into the bloodstream, and changes in incretin hormone activity also play important roles. From this point of view, the investigation of key molecular targets involved in glucose homeostasis is relevant for evaluating compounds with potential antidiabetic effects. Thus, the present in silico molecular docking study was carried out to explain the possible molecular basis of the hypoglycemic effect observed under experimental conditions. The selected targets, alpha-glucosidase and DPP-4, are directly involved in two important antidiabetic mechanisms: inhibition of intestinal carbohydrate hydrolysis and support of incretin-mediated insulin secretion. This approach provides additional molecular evidence for the experimentally observed hypoglycemic activity and helps to assess the antidiabetic potential of the studied compounds.

Alpha-glucosidase is considered one of the main enzymes involved in the hydrolysis of poly- and oligosaccharides into glucose in the intestine. A decrease in the activity of this enzyme reduces the rate at which carbohydrates are converted into glucose, which in turn makes it possible to limit the absorption of glucose from the intestine into the blood and to decrease the level of postprandial hyperglycemia. For this reason, alpha-glucosidase was selected as an important inhibitory target for evaluating antidiabetic activity. For the docking analysis of this enzyme, the crystal structure of human maltase-glucoamylase in complex with acarbose — PDB ID: 2QMJ — was used as the basis.

DPP-4 is an important enzyme that regulates the activity of the incretin system; it participates in the rapid degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Inhibition of DPP-4 activity prolongs the biological effect of GLP-1 in the blood, enhances glucose-dependent insulin secretion, suppresses glucagon release, and thereby contributes to the reduction of blood glucose levels. From this point of view, DPP-4 was selected as the second important target for evaluating antidiabetic activity at the molecular level. For this target, the crystal structure of human dipeptidyl peptidase-IV in complex with an inhibitor — PDB ID: 1X70 — was selected.

In the molecular docking analysis, L-carnitine, ammonium glycyrrhizinate, quercetin, and cysteine were selected as ligands. Their binding ability with alpha-glucosidase and DPP-4 protein targets was evaluated based on binding energy, conformational orientation within the active site, and interactions with amino acid residues, including hydrogen bonds, hydrophobic contacts, and other types of non-covalent interactions. This approach allows the possible molecular mechanisms underlying the antidiabetic effect observed under in vivo conditions to be clarified and provides additional support for the experimental findings.

Aim of the study: The aim of this study was to evaluate the possible antidiabetic mechanisms of the main components of the C-4 composition, including L-carnitine, ammonium glycyrrhizinate, quercetin, and cysteine, using in silico molecular docking analysis in an alloxan-induced experimental diabetes model. In this study, the binding energies, active-site orientations, and interactions of these compounds with amino acid residues of alpha-glucosidase and DPP-4 target proteins were analyzed.

Materials and methods. The computational part of the study was performed using freely available academic research tools. The three-dimensional structures of the antidiabetic target proteins were obtained from the Protein Data Bank (PDB), a widely used structural database for biological macromolecules. Based on their important roles in glucose metabolism and antidiabetic mechanisms, two target proteins were selected for molecular docking analysis: alpha-glucosidase, represented by human maltase-glucoamylase (PDB ID: 2QMJ), and dipeptidyl peptidase-IV (DPP-4, PDB ID: 1X70). Alpha-glucosidase was selected because this enzyme participates in the enzymatic hydrolysis of carbohydrates into glucose in the intestine. Inhibition of alpha-glucosidase may slow intestinal glucose absorption and reduce postprandial hyperglycemia. DPP-4 was selected due to its important role in the degradation of incretin hormones, particularly glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Inhibition of DPP-4 may prolong incretin hormone activity, enhance glucose-dependent insulin secretion, and contribute to the reduction of blood glucose levels.

L-carnitine, ammonium glycyrrhizinate, quercetin, and cysteine were selected as ligands for molecular docking analysis. The molecular structures of these compounds were prepared and optimized before docking. The target protein structures downloaded in PDB format were inspected using PyMOL. During receptor preparation, crystallographic water molecules, unnecessary heteroatoms, and other non-relevant components were removed from the protein structures. PyMOL was also used for visualization of receptor structures and post-docking analysis of ligand–protein interactions. Further preparation of receptors and ligands was carried out using AutoDock Tools. At this stage, polar hydrogen atoms were added to the protein structures, partial charges were assigned, rotatable bonds in ligands were defined, and the prepared receptor and ligand structures were converted into the required docking format. Molecular docking calculations were

performed using AutoDock 4.2 to determine the most favorable binding conformations of the studied ligands within the active regions of alpha-glucosidase and DPP-4.

For alpha-glucosidase docking, the grid box center was set at center_x = −21.417000, center_y = 7.904000, and center_z = −31.219399. For DPP-4 docking, the grid box center was set at center_x = 51.046000, center_y = 56.003000, and center_z = 44.224154. Docking results were evaluated based on binding free energy values expressed in kcal/mol, as well as hydrogen bonds, electrostatic interactions, hydrophobic contacts, π-interactions, and other non-covalent interactions formed between the ligands and amino acid residues of the target proteins. The obtained molecular docking results were used to support the possible antidiabetic mechanisms of the studied compounds at the molecular level. The binding results with alpha-glucosidase were interpreted in relation to the potential inhibition of intestinal carbohydrate hydrolysis and glucose absorption. The binding results with DPP-4 were interpreted in relation to the possible support of glucose-dependent insulin secretion through the incretin system. Thus, in silico molecular docking analysis provided additional molecular evidence for the hypoglycemic effect observed in the alloxan-induced experimental diabetes model.

Prior to molecular docking analysis, all target protein structures retrieved from the Protein Data Bank were subjected to a standardized preparation procedure, since raw PDB files are generally not directly suitable for docking calculations. The downloaded protein structures may contain crystallographic water molecules, co-crystallized ligands, ions, missing hydrogen atoms, and other unnecessary heteroatoms. If these components are not properly removed, they may affect the accuracy of docking results [10]. Therefore, all additional molecules that were not directly related to the target protein structure were removed before docking. In this study, two antidiabetic target proteins were used: human maltase-glucoamylase/alpha-glucosidase (PDB ID: 2QMJ) and dipeptidyl peptidase-IV (DPP-4, PDB ID: 1X70). These proteins were selected because of their important roles in glucose metabolism and antidiabetic mechanisms. Alpha-glucosidase is involved in the enzymatic breakdown of carbohydrates into glucose in the intestine, whereas DPP-4 plays an important role in the degradation of incretin hormones such as GLP-1 and GIP.

During receptor preparation, crystallographic water molecules, unnecessary heteroatoms, ions, and other non-protein components were removed from the structures. Co-crystallized ligands were also deleted before docking calculations. When necessary, the original ligands present in the crystal structures were saved separately and used as reference molecules for comparison or docking validation. The removal of these additional components is important because they may occupy the ligand-binding pocket, partially block the active site, create artificial steric hindrance, or produce non-physiological interactions with the tested ligands. The prepared receptor structures were visually inspected using PyMOL. At this stage, the structural integrity of the proteins and the condition of their active or functionally important regions were evaluated [11]. No major structural defects that could significantly affect the docking analysis were observed in the selected receptor structures. After visual inspection, the protein structures were imported into AutoDock Tools for further preparation. Polar hydrogen atoms were added to the receptors, and Kollman united atom charges were assigned to properly represent the electrostatic properties of the target proteins. Protonation states were adjusted according to the standard AutoDock Tools preparation workflow to approximate physiological conditions. The final prepared receptor structures were saved in .pdbqt format, which contains atom type information and partial charges required for AutoDock docking calculations. For alpha-glucosidase docking, the grid box center was set at center_x =

−21.417000, center_y = 7.904000, and center_z = −31.219399. For DPP-4 docking, the grid box center was set at center_x = 51.046000, center_y = 56.003000, and center_z = 44.224154.

Ligand structures were prepared using a similar protocol [12]. The selected compounds, including L-carnitine, ammonium glycyrrhizinate, quercetin, and cysteine, were imported into AutoDock Tools. Hydrogen atoms were added to the ligands, Gasteiger charges were assigned, and non-polar hydrogen atoms were merged according to the standard AutoDock procedure. Rotatable bonds and root atoms were automatically identified by AutoDock Tools and manually checked when necessary to ensure proper ligand flexibility during docking. Finally, all prepared ligand structures were saved in .pdbqt format and used for molecular docking simulations. These preparation steps ensured that the docking results reflected the direct interactions between the selected antidiabetic target proteins, alpha-glucosidase and DPP-4, and the studied compounds. The resulting ligand–protein complexes were analyzed based on binding free energy, hydrogen bonds, electrostatic interactions, hydrophobic contacts, π-interactions, and other non-covalent interactions formed within the active site.

Results

L-carnitine

L-carnitine molecule was subjected to molecular docking analysis with the alpha-glucosidase target protein. During the docking procedure, the active site coordinates were set as center_x = −21.417000, center_y = 7.904000, and center_z = −31.219399. According to the obtained results, L-carnitine bound to the active site of alpha-glucosidase in an energetically favorable position and showed a binding energy of −4.7 kcal/mol. The negative value of the binding energy indicates that the formation of the ligand–protein complex is thermodynamically favorable. The binding energy of −4.7 kcal/mol suggests that L-carnitine has a moderate binding affinity toward alpha-glucosidase. This result indicates that L-carnitine may partially suppress alpha-glucosidase activity, thereby potentially slowing the breakdown of carbohydrates into glucose. The 2D interaction analysis showed that L-carnitine interacted with several amino acid residues within the active site of alpha-glucosidase. In particular, conventional hydrogen bond and carbon hydrogen bond interactions were observed with ASP A:772 and TRP A:290 residues. In addition, electrostatic attractive charge interactions were formed with GLU A:774 and ARG A:748 residues. These interactions may contribute to the relatively stable positioning of the ligand within the active site of the enzyme.

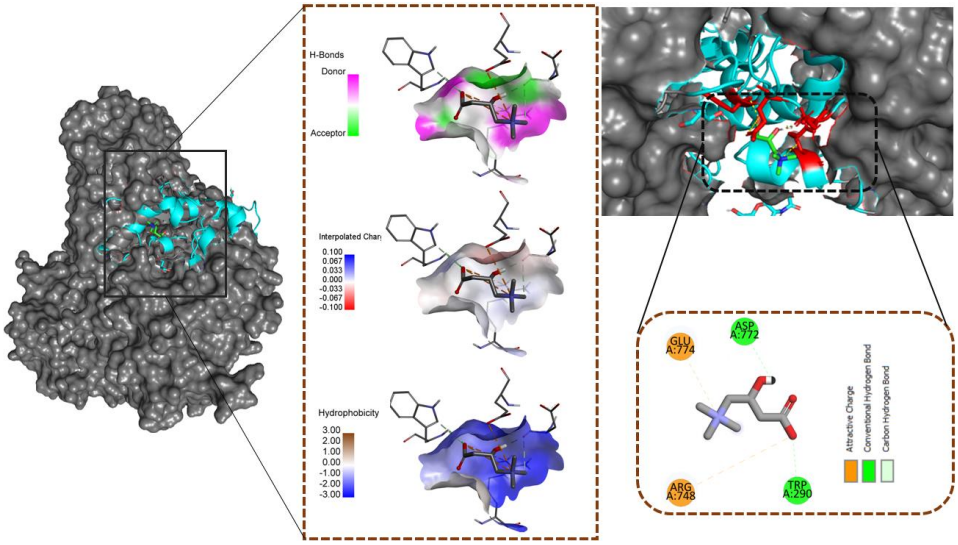


Figure 1. Molecular docking results of L-carnitine with alpha-glucosidase

These results indicate that L-carnitine has the ability to interact with the alpha-glucosidase target. Since alpha-glucosidase plays an important role in the intestinal breakdown of carbohydrates into glucose, inhibition of this enzyme may contribute to the reduction of postprandial hyperglycemia. From this point of view, the interaction of L-carnitine with alpha-glucosidase can be considered as an additional molecular basis for its possible antidiabetic mechanism. In the molecular docking analysis performed with the DPP-4 target protein, the binding properties of L-carnitine within the active site of this enzyme were evaluated. For docking, the active site coordinates were set as center_x = 51.046000, center_y = 56.003000, and center_z = 44.224154. According to the calculation results, L-carnitine showed a binding energy of −4.8 kcal/mol with the DPP-4 enzyme. The negative binding energy indicates that the formation of the ligand–protein complex between L-carnitine and DPP-4 is energetically favorable. The value of −4.8 kcal/mol reflects a moderate binding affinity of this molecule toward the active site of DPP-4. This finding suggests that L-carnitine may partially suppress DPP-4 enzyme activity and thereby participate in antidiabetic mechanisms related to the incretin system.

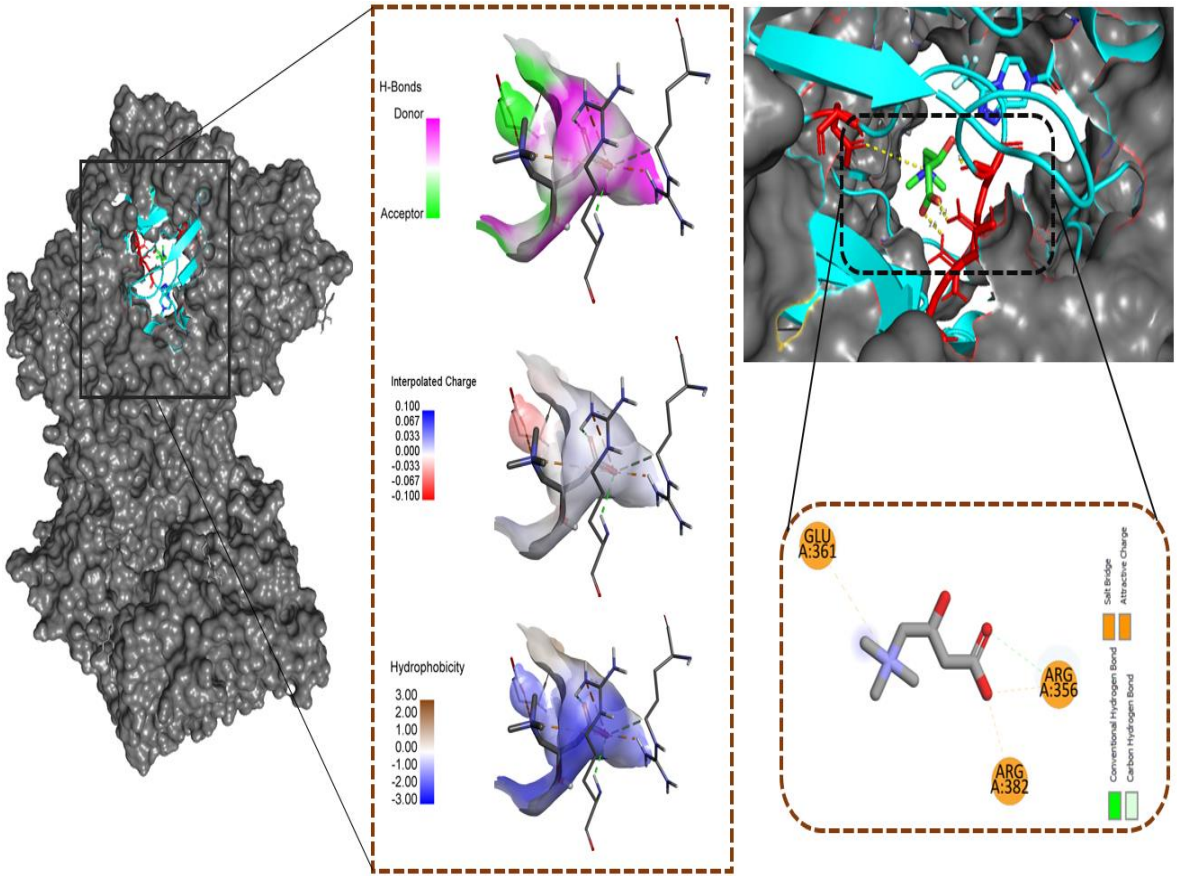


Figure 2. Molecular docking results of L-carnitine with the DPP-4 enzyme

The 2D interaction analysis showed that L-carnitine interacted with GLU A:361, ARG A:356, and ARG A:382 amino acid residues within the active site of DPP-4. In particular, salt bridge and attractive charge electrostatic interactions were observed with GLU A:361 and ARG A:382 residues. In addition, conventional hydrogen bond and carbon hydrogen bond interactions were formed with ARG A:356. These interactions may contribute to the relatively stable conformation of L-carnitine within the active site of the enzyme. DPP-4 plays an important role in the rapid degradation of incretin hormones such as GLP-1 and GIP. Therefore, inhibition of this enzyme may prolong the biological activity of GLP-1, support glucose-dependent insulin secretion, and contribute to the reduction of blood glucose levels. From this point of view, the

binding of L-carnitine with DPP-4 can be considered as additional molecular evidence supporting its possible involvement in the antidiabetic mechanism through the incretin system.

Ammonium glycyrrhizinate

Molecular docking analysis was performed between ammonium glycyrrhizinate and the alpha-glucosidase target protein. During the docking procedure, the active site coordinates of the enzyme were set as center_x = −21.417000, center_y = 7.904000, and center_z = −31.219399. According to the obtained results, ammonium glycyrrhizinate showed a binding energy of −10.2 kcal/mol with alpha-glucosidase. The binding energy value of −10.2 kcal/mol indicates that this ligand has a high binding affinity toward the active site of alpha-glucosidase. This value was considerably stronger than that obtained for L-carnitine, suggesting that ammonium glycyrrhizinate may have a higher potential to inhibit alpha-glucosidase enzyme activity.

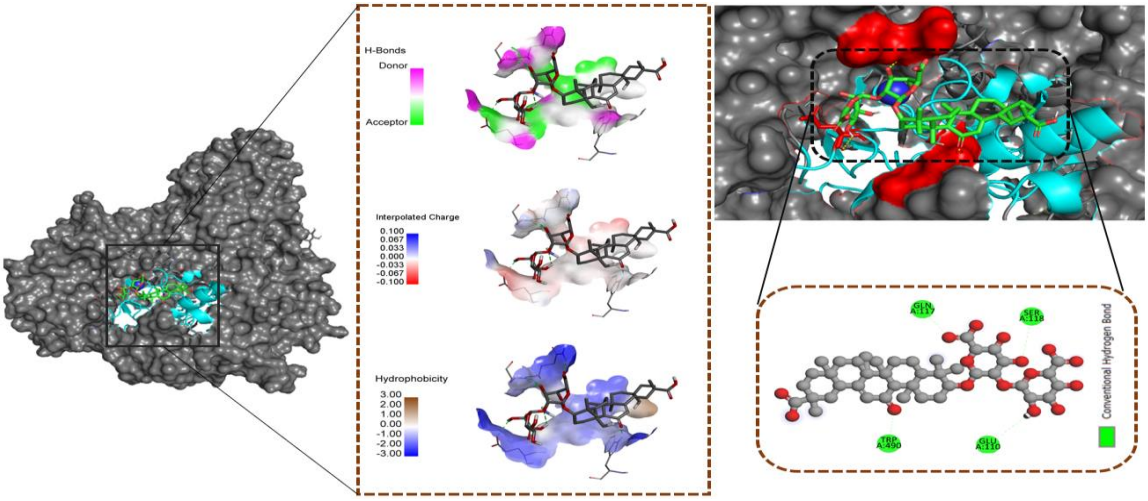


Figure 3. Molecular docking results of ammonium glycyrrhizinate with alpha-glucosidase

The 2D interaction analysis showed that the ligand interacted with several important amino acid residues within the active site of alpha-glucosidase. In particular, conventional hydrogen bond interactions were formed with GLN A:117, SER A:118, TRP A:490, and GLU A:110 residues. The presence of hydrogen bonds may contribute to the stabilization of the ligand–protein complex and ensure a stable positioning of the ligand within the active site.

Since ammonium glycyrrhizinate contains several hydroxyl and carboxyl groups, it has the ability to form multiple hydrogen bonds with amino acid residues in the active site of alpha-glucosidase. This may explain its stronger binding with the enzyme and suggests a possible ability to reduce enzymatic activity. Alpha-glucosidase is an important enzyme involved in the breakdown of carbohydrates into glucose in the intestine. Inhibition of this enzyme may slow the conversion of carbohydrates into glucose, reduce glucose absorption into the bloodstream, and help decrease postprandial hyperglycemia. From this point of view, the strong binding of ammonium glycyrrhizinate with alpha-glucosidase may play an important role in its antidiabetic mechanism.

Molecular docking analysis was also performed between ammonium glycyrrhizinate and the DPP-4 target protein. During the docking procedure, the active site coordinates of the DPP-4 enzyme were set as center_x = 51.046000, center_y = 56.003000, and center_z = 44.224154. According to the calculation results, ammonium glycyrrhizinate showed a binding energy of −13.2 kcal/mol with DPP-4. The highly negative binding energy indicates that the complex between the ligand and DPP-4 is energetically very stable. The value of −13.2 kcal/mol demonstrates that

ammonium glycyrrhizinate has a strong binding affinity toward the active site of DPP-4. This result suggests that this compound may have a high potential to inhibit DPP-4 enzyme activity.

The 2D interaction analysis showed that the ligand interacted with several amino acid residues within the active site of DPP-4. In particular, conventional hydrogen bond interactions were observed with SER A:212, TRP A:305, and ALA A:306 residues. In addition, a carbon hydrogen bond interaction was formed with THR A:304. These interactions may contribute to the stable positioning of the ligand within the active site of the enzyme and support the stabilization of the ligand–protein complex. At the same time, an unfavorable acceptor–acceptor interaction was observed with PHE A:364. However, the overall binding energy of −13.2 kcal/mol indicates that this unfavorable interaction did not significantly reduce the general stability of the ligand binding. On the contrary, the presence of multiple hydrogen bonds and other stabilizing interactions may have allowed the ligand to remain strongly bound within the active site of DPP-4.

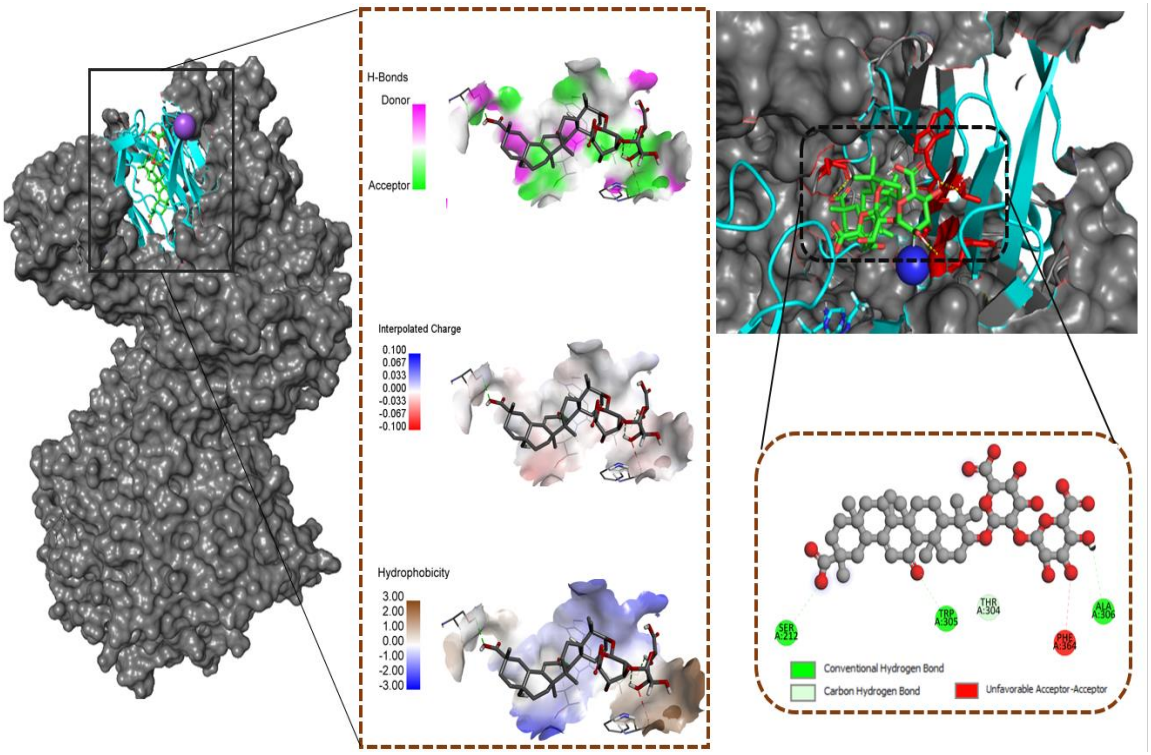


Figure 4. Molecular docking results of ammonium glycyrrhizinate with the DPP-4 enzyme

Quercetin

Evaluation of the interaction between quercetin and alpha-glucosidase showed that this compound had a high binding tendency toward the active site of the enzyme. Docking was performed using the coordinates center_x = −21.417000, center_y = 7.904000, and center_z = −31.219399. The calculated binding energy for quercetin was −8.4 kcal/mol, indicating that the ligand was positioned in a stable conformation within the active region of alpha-glucosidase. The polyphenolic nature of quercetin played an important role in its interaction with the enzyme. The hydroxyl groups present in the molecular structure enabled the formation of several hydrogen bonds with amino acid residues located in the active site of the enzyme. In particular, conventional hydrogen bond interactions were observed with GLU A:510, LYS A:776, GOL A:3002, and LEU A:286 residues. These interactions may play an important role in maintaining quercetin within the active site and stabilizing the ligand–protein complex.

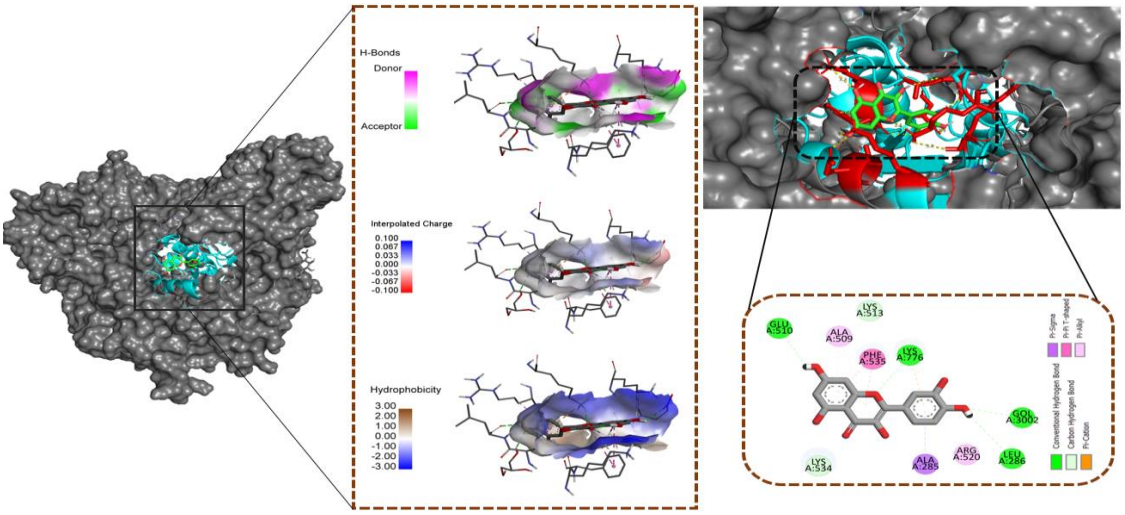


Figure 5. Molecular docking results of quercetin with alpha-glucosidase

At the same time, the aromatic rings present in the quercetin molecule contributed to the formation of additional π-type interactions within the active site of the enzyme. In particular, π-sigma interaction was observed with ALA A:285, π–π T-shaped interaction with PHE A:535, and π-alkyl interactions with ALA A:509 and ARG A:520 residues. The π-cation interaction recorded with LYS A:776 may further contribute to the stronger stabilization of the ligand within the enzyme pocket. In addition, carbon hydrogen bond interactions were also detected with LYS A:513 and LYS A:534 residues. The obtained results indicate that quercetin binds to the active site of alpha-glucosidase through several types of interactions rather than through a single interaction mode. The combined participation of hydrogen bonds, π-interactions, and hydrophobic contacts may enhance the stability of the ligand–protein complex. Therefore, the binding energy of −8.4 kcal/mol was considered an important molecular indicator of the potential ability of quercetin to inhibit the alpha-glucosidase enzyme. The molecular docking results of quercetin with the DPP-4 enzyme made it possible to evaluate the possible involvement of this compound in antidiabetic mechanisms related to the incretin system. The docking calculation was performed using the active site coordinates of DPP-4: center_x = 51.046000, center_y = 56.003000, and center_z = 44.224154. As a result, a binding energy of −7.6 kcal/mol was recorded for quercetin. This value indicates that quercetin has a noticeable binding tendency toward the active site of DPP-4. The binding energy of −7.6 kcal/mol suggests the possible formation of a stable complex between the ligand and the enzyme. This result indicates that quercetin may reduce the degradation of incretin hormones such as GLP-1 and GIP by suppressing DPP-4 enzyme activity.

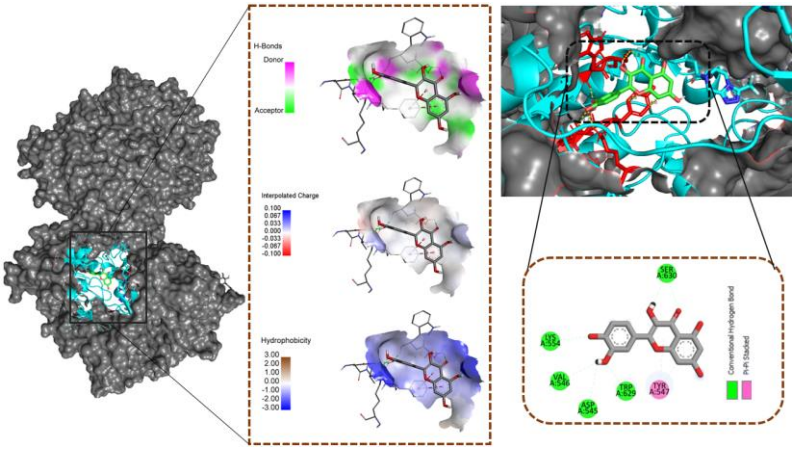


Figure 6. Molecular docking results of quercetin with the DPP-4 enzyme

In the 2D docking map, quercetin was shown to interact with several important amino acid residues within the active site of DPP-4. In particular, conventional hydrogen bond interactions were observed with LYS A:554, VAL A:546, ASP A:545, TRP A:629, and SER A:630 residues. These hydrogen bonds may be important for the specific orientation of quercetin within the active region of the enzyme and for maintaining the stability of the ligand–protein complex. In addition, the aromatic rings of quercetin formed a π–π stacked interaction with the TYR A:547 residue. This type of interaction is especially important for polyphenolic compounds, as it may contribute to the formation of stronger contacts with the hydrophobic regions of the enzyme. Therefore, both hydrogen bonds and aromatic π-interactions were involved in the binding of quercetin to DPP-4.

Cysteine

The interaction of cysteine with the alpha-glucosidase enzyme was also evaluated using molecular docking analysis. Docking was performed based on the active site coordinates of alpha-glucosidase: center_x = −21.417000, center_y = 7.904000, and center_z = −31.219399. The calculated binding energy for cysteine was −4.0 kcal/mol. This result indicates that cysteine is able to interact with the active site of alpha-glucosidase; however, its binding strength was lower compared with quercetin and ammonium glycyrrhizinate. The binding energy value of −4.0 kcal/mol suggests that the ligand–protein complex can be formed, although the interaction appears to be relatively weak or moderate.

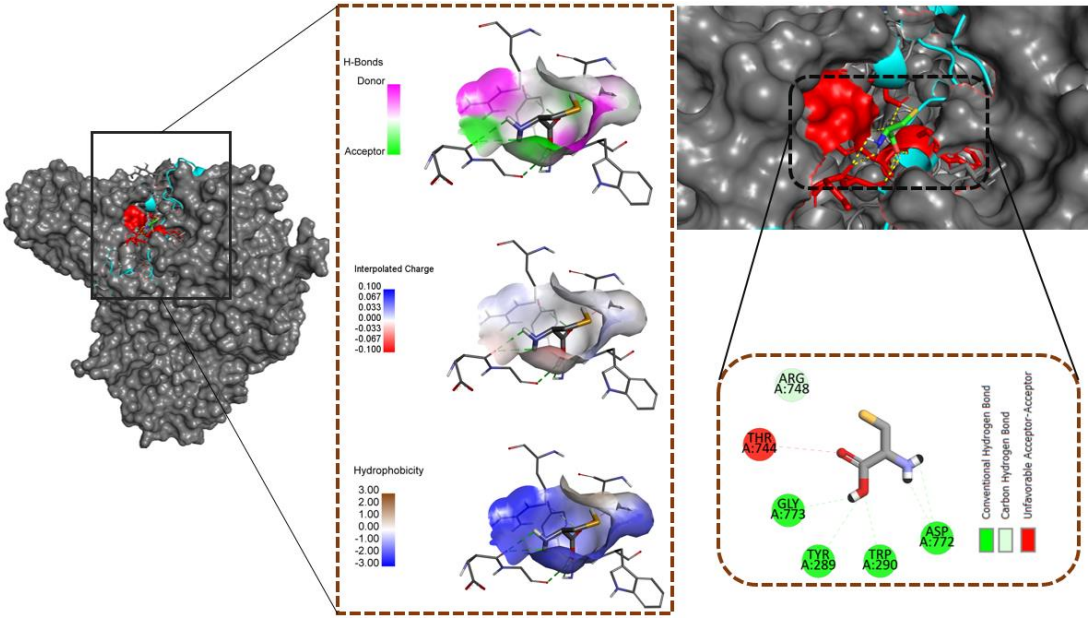


Figure 7. Molecular docking results of cysteine with alpha-glucosidase

The 2D interaction map showed that cysteine formed contacts with several amino acid residues within the active site of alpha-glucosidase. In particular, conventional hydrogen bond interactions were observed with ASP A:772, GLY A:773, TYR A:289, and TRP A:290 residues. In addition, a carbon hydrogen bond interaction was recorded with ARG A:748. These interactions may help cysteine to maintain a temporarily stable position within the active region of the enzyme. At the same time, an unfavorable acceptor–acceptor interaction was detected with THR A:744. This type of interaction may partially limit the overall stability of the ligand–enzyme complex. Therefore, the binding energy of −4.0 kcal/mol obtained for cysteine indicates that, although this compound is able to bind to alpha-glucosidase, its inhibitory potential is not strong. The interaction of cysteine with the DPP-4 enzyme was also evaluated based on the active region where quercetin was bound. The calculated binding energy for cysteine was −4.1 kcal/mol. This value indicates

that cysteine has the ability to interact with the active site of DPP-4; however, its binding strength is considerably lower than that of quercetin.

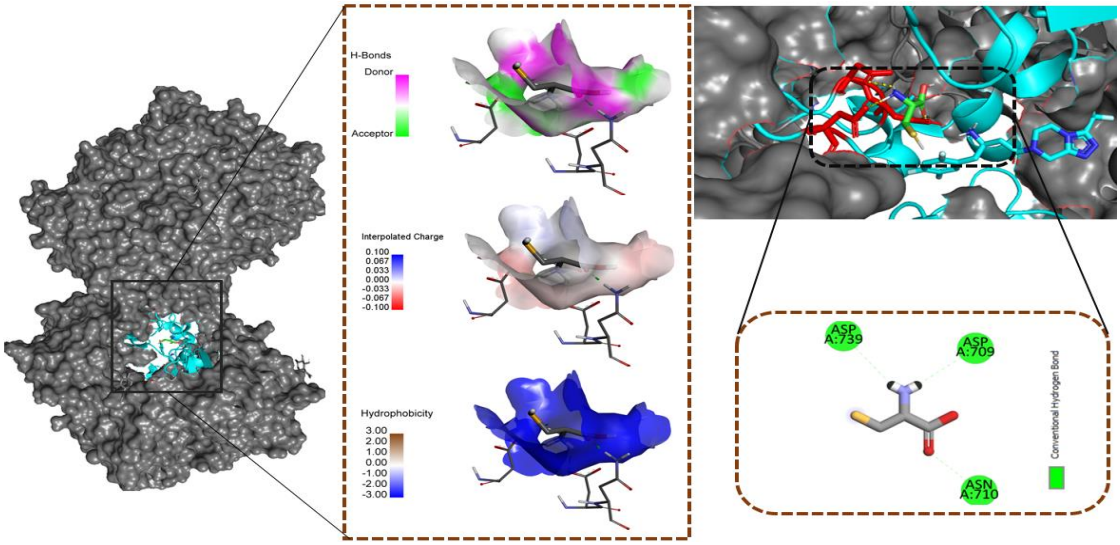


Figure 8. Molecular docking results of cysteine with the DPP-4 enzyme

According to the 2D docking analysis, cysteine interacted with the active region of DPP-4 mainly through hydrogen bonds. The ligand formed conventional hydrogen bond interactions with ASP A:739, ASP A:709, and ASN A:710 residues. These interactions may help cysteine to remain within the active region of DPP-4 to a certain extent. However, due to its small molecular structure, cysteine was unable to form a broad contact surface within the active site of DPP-4. While the aromatic rings and multiple hydroxyl groups of quercetin allowed the formation of several types of interactions with DPP-4, cysteine mainly showed limited hydrogen bond interactions. Therefore, the binding energy of −4.1 kcal/mol reflects a weak to moderate binding tendency of cysteine toward DPP-4. Since DPP-4 is involved in the degradation of incretin hormones such as GLP-1 and GIP, inhibition of this enzyme is important in the mechanism of antidiabetic activity. However, the docking result obtained for cysteine indicates that this compound is not the main strong inhibitor of DPP-4. Nevertheless, the formation of hydrogen bonds between cysteine and DPP-4 suggests that it may participate as an auxiliary molecular factor in the overall antidiabetic effect.

Discussion

The present in silico study was performed to support and explain the possible molecular mechanisms underlying the antidiabetic activity of the C-4 composition. Molecular docking analysis was carried out using two important antidiabetic target proteins: alpha-glucosidase and dipeptidyl peptidase-IV (DPP-4). These targets were selected because they are directly involved in two key mechanisms of blood glucose regulation. Alpha-glucosidase participates in the intestinal hydrolysis of carbohydrates into glucose, while DPP-4 is responsible for the rapid degradation of incretin hormones such as GLP-1 and GIP. Therefore, inhibition of these enzymes may contribute to the reduction of hyperglycemia through different but complementary pathways. The docking results demonstrated that the studied compounds interacted with both alpha-glucosidase and DPP-4 with different binding affinities. Among all investigated ligands, ammonium glycyrrhizinate showed the strongest binding toward both targets. Its binding energy with alpha-glucosidase was −10.2 kcal/mol, while its interaction with DPP-4 showed an even stronger binding energy of −13.2 kcal/mol. These values indicate a high molecular affinity of ammonium glycyrrhizinate toward the active sites of both enzymes. The

strong binding may be explained by the presence of several hydroxyl and carboxyl groups in its structure, which allow the formation of multiple hydrogen bonds and stabilizing interactions with amino acid residues of the target proteins. This suggests that ammonium glycyrrhizinate may be one of the main components responsible for the antidiabetic potential of the C-4 composition. Quercetin also demonstrated notable binding activity against both antidiabetic targets. The binding energy of quercetin with alpha-glucosidase was −8.4 kcal/mol, while its binding energy with DPP-4 was −7.6 kcal/mol. These results suggest that quercetin may contribute to the antidiabetic effect through dual interaction with carbohydrate-digesting and incretin-regulating enzymes. The interaction profile of quercetin was characterized by hydrogen bonds, π-interactions, and hydrophobic contacts. Such interactions are consistent with the polyphenolic nature of quercetin and may support the stable positioning of the ligand within the active sites of the enzymes. Therefore, quercetin can be considered an important active component that may support the hypoglycemic effect of the C-4 composition. In comparison, L-carnitine and cysteine showed lower binding energies with both target proteins. L-carnitine demonstrated binding energies of −4.7 kcal/mol with alpha-glucosidase and −4.8 kcal/mol with DPP-4. Cysteine showed binding energies of −4.0 kcal/mol and −4.1 kcal/mol with alpha-glucosidase and DPP-4, respectively. Although these values indicate that both compounds are able to interact with the selected targets, their binding strength was weaker than that of ammonium glycyrrhizinate and quercetin. This suggests that L-carnitine and cysteine may not act as the main enzyme-inhibiting components of the composition. However, their presence may still be biologically relevant, since they may contribute to the overall antidiabetic effect through additional metabolic, antioxidant, or supportive mechanisms. The stronger binding of ammonium glycyrrhizinate and quercetin to alpha-glucosidase suggests a possible mechanism related to the inhibition of intestinal carbohydrate hydrolysis. Suppression of alpha-glucosidase activity can slow the conversion of complex carbohydrates into glucose, reduce glucose absorption into the bloodstream, and decrease postprandial hyperglycemia. This mechanism is especially important in diabetes mellitus, where control of postprandial glucose elevation plays a significant role in maintaining glucose homeostasis.

The interaction of the studied compounds with DPP-4 provides another possible mechanism for their antidiabetic activity. DPP-4 inhibition may prolong the biological activity of incretin hormones, particularly GLP-1 and GIP. As a result, glucose-dependent insulin secretion may be enhanced, glucagon release may be reduced, and blood glucose levels may decrease. The high binding affinity of ammonium glycyrrhizinate and the moderate-to-strong binding of quercetin with DPP-4 suggest that these compounds may support incretin-mediated glucose regulation.

Overall, the molecular docking results indicate that the antidiabetic potential of the C-4 composition may be associated with multiple molecular mechanisms. The most important contribution appears to be related to ammonium glycyrrhizinate and quercetin, which showed the strongest interactions with both alpha-glucosidase and DPP-4. L-carnitine and cysteine showed weaker direct binding to the selected targets, but they may act as auxiliary components that support the general biological activity of the composition.

These findings provide additional molecular evidence for the hypoglycemic effect observed in the alloxan-induced experimental diabetes model. The in silico results suggest that the C-4 composition may exert its antidiabetic activity not through a single pathway, but through a combination of mechanisms, including reduced intestinal glucose absorption and

support of incretin-mediated insulin secretion. However, molecular docking is a predictive computational method, and the obtained results should be interpreted as supportive evidence. Further experimental studies, including enzyme inhibition assays and additional pharmacological investigations, are required to confirm the direct inhibitory effects of these compounds on alpha-glucosidase and DPP-4.

Conclusion

The molecular docking results showed that the studied compounds had different degrees of binding ability with alpha-glucosidase and DPP-4 target proteins. The negative binding energy values observed for both targets confirm that ligand–protein complexes were formed in an energetically favorable manner. At the same time, the differences in binding energies indicated that the molecular affinities of the compounds toward these enzymes were not the same.

According to the docking results with alpha-glucosidase, the strongest binding was observed for ammonium glycyrrhizinate. This compound bound to the active site of alpha-glucosidase with a binding energy of −10.2 kcal/mol, suggesting its high potential to inhibit the enzyme. Quercetin also demonstrated relatively strong binding to this target, with a binding energy of −8.4 kcal/mol. In contrast, L-carnitine and cysteine showed binding energies of −4.7 and −4.0 kcal/mol, respectively, indicating that although they are able to interact with alpha-glucosidase, their inhibitory potential may be lower.The results obtained for the DPP-4 target also showed the highest binding ability for ammonium glycyrrhizinate. This compound bound to the active site of DPP-4 with a binding energy of −13.2 kcal/mol, indicating a strong molecular affinity toward this enzyme. Quercetin showed a binding energy of −7.6 kcal/mol with DPP-4, suggesting its possible involvement in antidiabetic mechanisms related to the incretin system. L-carnitine and cysteine demonstrated binding energies of −4.8 and −4.1 kcal/mol, respectively, indicating that these compounds can interact with DPP-4, although their binding strength is relatively lower.

In the overall comparison, ammonium glycyrrhizinate demonstrated the strongest binding energies with both target proteins, alpha-glucosidase and DPP-4. This finding indicates that this compound may be an important component in the antidiabetic mechanism of action. Quercetin was evaluated as the second most active ligand, as it formed stable interactions with both enzymes. In contrast, L-carnitine and cysteine showed relatively lower binding energies, suggesting that their effects may be associated more with additional metabolic, antioxidant, or supportive biological mechanisms rather than with a primary inhibitory mechanism.

Based on the molecular docking results, ammonium glycyrrhizinate and quercetin were identified as the main components with higher potential to inhibit alpha-glucosidase and DPP-4 among the studied compounds. Inhibition of alpha-glucosidase may slow the intestinal breakdown of carbohydrates into glucose and reduce postprandial hyperglycemia. Inhibition of DPP-4 may prolong the activity of incretin hormones such as GLP-1 and GIP, thereby supporting glucose-dependent insulin secretion. From this perspective, the in silico results provide a molecular basis for the hypoglycemic effect observed under experimental conditions.

Thus, molecular docking analysis showed that the antidiabetic activity of the components of the C-4 composition may be mediated through several mechanisms. In particular, the strong binding of ammonium glycyrrhizinate and quercetin with alpha-glucosidase and DPP-4 suggests that these compounds may play an important molecular role

in reducing blood glucose levels. L-carnitine and cysteine, on the other hand, may be considered auxiliary components that support the overall antidiabetic effect.

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