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In Silico Study to Evaluate the Inhibitory Activity of a Few Phenylethanoid Glycosides on GSK3-β Protein for Faster Diabetic Wound Healing †

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Abstract: Chronic wound resulting from Diabetes Mellitus is a significant cause of amputation worldwide. Secondary infections, lowering of nitric oxide synthase level, reduction of glucose-6-phosphate dehydrogenase levels, improper extracellular matrix remodelling, neuropathy, abnormality of endothelial cell function, and vasculopathy impedes the normal wound healing cycle during diabetes. Multiple studies have concluded that Ser9 phosphorylation causes inhibition of the glycogen synthase kinase-3β (GSK3-β) protein, which is essential for faster diabetic wound healing. Hence this protein could be a potential target for molecular interactions with prospective wound-healing molecules. Verbascoside, martynoside, echinacoside, crenatoside, and salidroside are a few phenylethanoid glycosides that have the potential wound-healing ability by increasing extracellular matrix synthesis, angiogenesis, keratinocyte migration, and the functioning of macrophages and neutrophils. Thus, the five glycosides were subjected to molecular docking with GSK3-β protein (PDB ID: 1I09). This study revealed strong binding interactions with GSK3-β (between −10.2 to −7.3 kcal/mol) and inhibition constants (between 0.032 to 4.397 μM) which suggested potent inhibition of the target protein even at lower concentrations of these compounds. Further, the docked complexes were visualized to find the interaction of the ligands with the amino acid residues. However, further in-vivo and in-vitro studies are required to validate the activity of these phenylpropanoid glycosides in diabetic wound healing.

Keywords: phenylpropanoid glycosides; diabetes mellitus; wound healing; binding interactions; inhibition constants

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1. Introduction

The most common metabolic disorder worldwide is Diabetes Mellitus (DM). The most common complication of this disorder is a diabetic foot ulcer or diabetic wound. Associated complications such as neuropathy, secondary infection, reduced levels of glucose-6-phosphate dehydrogenase, decreased nitric oxide synthase levels, improper remodelling of extracellular matrix, and abnormal vasculopathy delay the wound healing process [1]. One primary target to fasten the wound healing process is the glycogen synthase kinase-3β (GSK3-β) which follows the Activated Phosphatidylinositol 3 Kinase/Protein Kinase B signalling pathway or PI3K/AKT pathway [1,2]. This target protein is involved in cellular inflammation, migration, and metabolism. Several studies have revealed that phosphorylation of the Ser9 position via the PI3K/AKT pathway leads to the downregulation of the GSK3-β protein, which is required for chronic wound healing [3]. The phosphorylated protein is found to increase collagen production, reduce apoptosis,

and increase migration [2,3]. Currently, extensive research is being conducted on phenylethanoid glycosides (PhGs). These compounds have shown antimicrobial, antioxidant, antidiabetic, cardioprotective, neuroprotective, and wound-healing activity. Common phenylethanoid glycosides are verbascoside, martynoside, salidroside, crenatoside, echinacoside, and forsythoside [4,5].

2. Materials and Methods

The in silico study was done by the following steps:

1. Acquisition of the target protein—The three-dimensional glycogen synthase kinase-3β (GSK3-β) structure (PDB ID: 1I09) was obtained from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank [6].
2. Selection of ligand—The three-dimensional structures of Verbascoside, Echinacoside, Martynoside, Salidroside, and Crenatoside were obtained PubChem database.
3. Active site of target protein analysis—PyMOL software was used to analyze the target binding site of the receptor protein.
4. Molecular docking—The target protein was prepared by AutoDock Tools. The grid box on the active binding site was generated for docking. The prepared protein and the ligands were docked using AutoDock Vina based on the scoring function [7].
5. Analysis of docked confirmations—The docked conformations with the minimum binding energies for each ligand was analyzed by LigPlot+ [8] and PyMOL software to view the hydrogen bonds, hydrophobic bonds, and ionic interactions.

3. Results

After successful docking with the protein, the minimum binding energy was obtained for every ligand. The docked conformations and the binding sites were visualized for hydrogen bonding, hydrophobic, and ionic interactions. The inhibition constants (K_i) were calculated from the formula mentioned below,

$$K_i = \exp(\Delta G/RT)$$

Here ΔG indicates the minimum binding energies of the docked conformations, R indicates the universal gas constant ($R = 1.985 \times 10^{-3}$ kcal mol^{-1} K^{-1}), and absolute temperature is indicated by T (T = 298.15 K).

Table 1. The estimated inhibition constants and the minimum binding energies of the ligands docked with GSK3-β.

Ligands	Estimated Free Binding Energy (kcal/mol)	Estimated Inhibition Constant (K_i) (μM)
Crenatoside	−10.2	0.032
Echinacoside	−9.2	0.177
Verbascoside	−8.7	0.412
Martynoside	−8.1	1.137
Salidroside	−7.3	4.397

The docking results were studied through LigPlot+ software to give a two-dimensional view of the hydrogen bonding and the hydrophobic interactions between the ligands and the residues of the binding site of the protein molecule. The three-dimensional visualization of the docking results was carried out using PyMOL software.

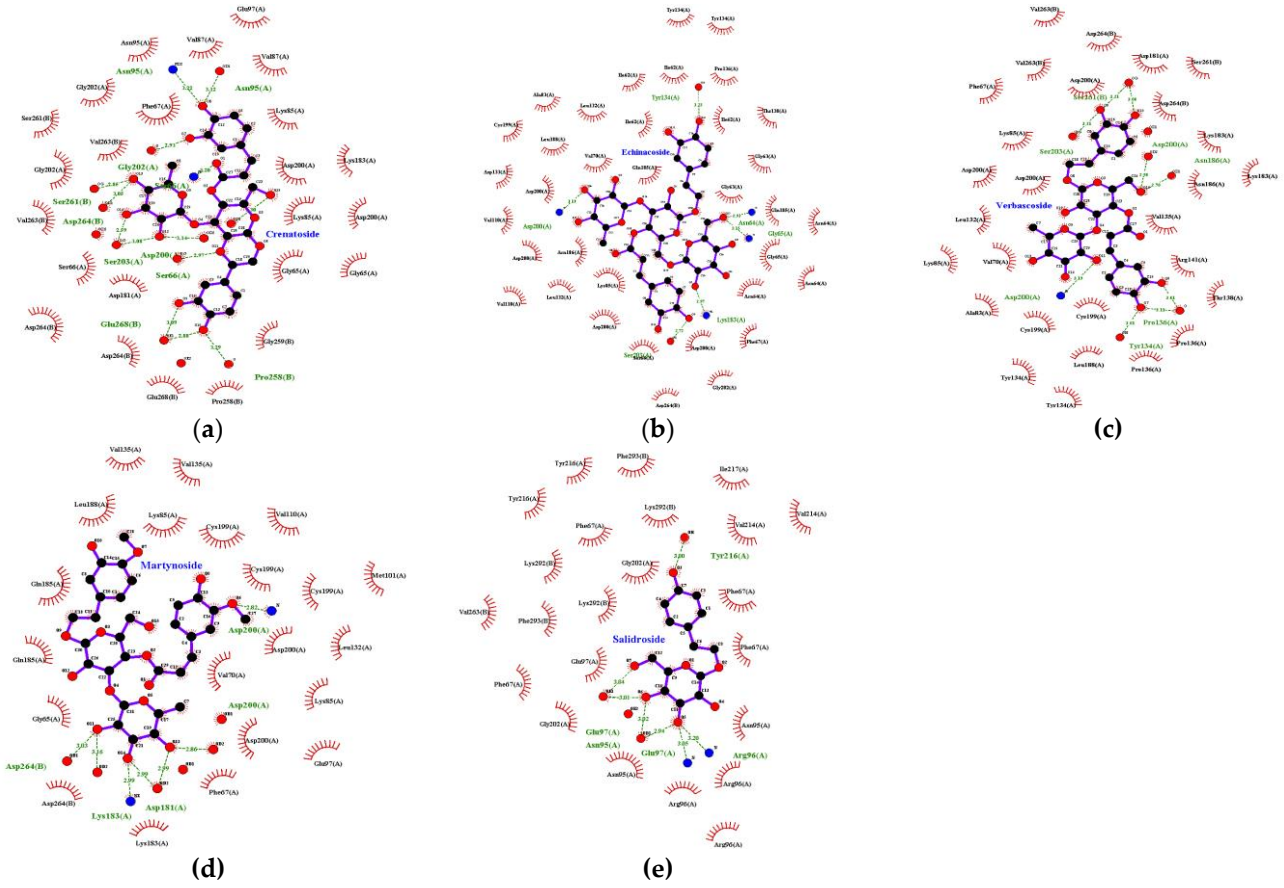

Figure 1. The two-dimensional representation of the docked ligand's hydrophobic interactions and hydrogen bonds in the target's binding site. (**a**) Crenatoside; (**b**) Echinacoside; (**c**) Verbascoside; (**d**) Martynoside; (**e**) Salidroside.

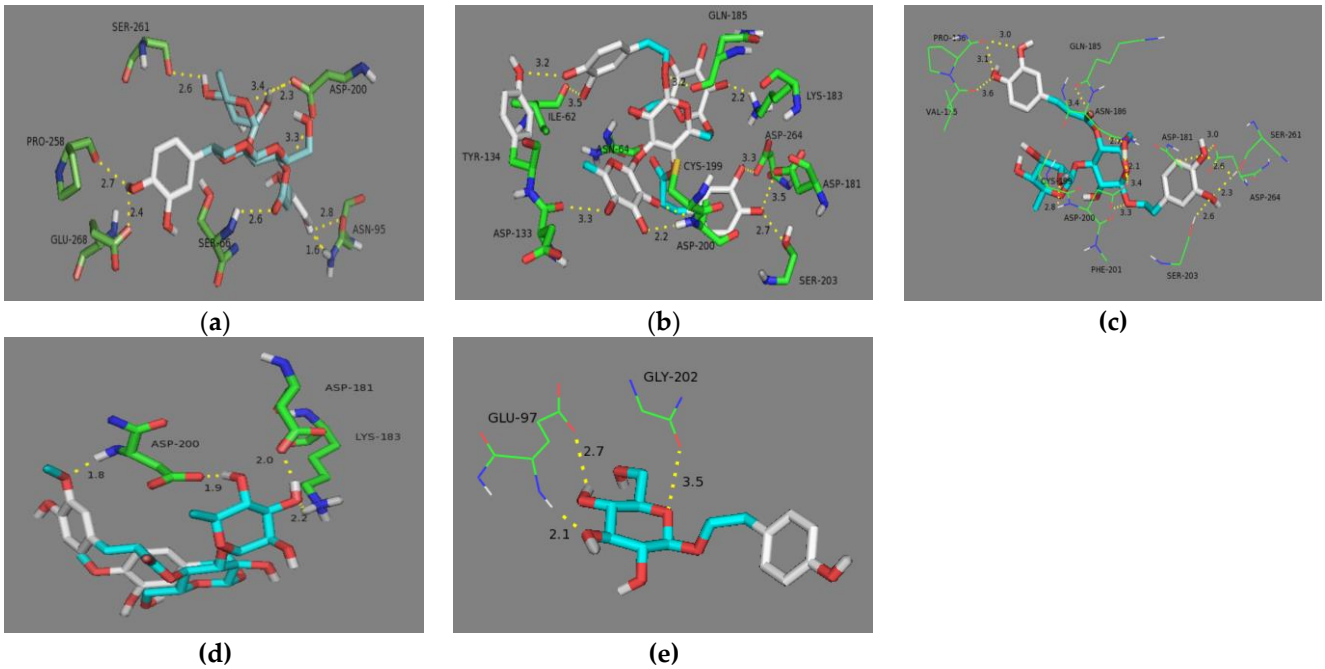

Figure 2. The three-dimensional representation of the active binding site of the protein involved in ionic interactions with the ligands. (**a**) Crenatoside; (**b**) Echinacoside; (**c**) Verbascoside; (**d**) Martynoside; (**e**) Salidroside.

Table 2. The hydrophobic interactions and hydrogen bonds of the ligands with the residues of the active binding site.

Ligands	Hydrogen Bonds	Hydrophobic Interactions
Crenatoside	Ser66, Asn95, Asp200, Gly202, Ser203, Ser261, Asp264, Glu268	Gly65, Ser66, Phe67, Lys85, Val87, Asn95, Glu97, Asp181, Lys183, Asp200, Gly202, Gly259, Ser261, Val263, Asp264
Echinacoside	Asn64, Gly65, Lys183, Ser203	Ile62, Gly63, Asn64, Gly65, Ser66, Phe67, Ala83, Lys85, Val110, Leu132, Asp133, Tyr134, Pro136, Thr138, Val170, Gln185, Asn186, Leu188, Cys199, Asp200, Gly202, Asp264
Verbascoside	Tyr134, Pro136, Asn186, Asp200, Ser203, Ser261	Phe67, Val70, Ala83, Lys85, Leu132, Tyr134, Val135, Pro136, Thr138, Arg141, Asp181, Lys183, Asn186, Leu188, Cys199, Asp200, Ser261, Val263, Asp264
Martynoside	Asp181, Lys183, Asp200, Asp264	Gly65, Phe67, Val70, Lys85, Glu97, Met101, Val110, Leu132, Val135, Lys183, Gln185, Leu188, Cys199, Asp200, Asp264
Salidroside	Asn95, Arg96, Glu97, Tyr216	Phe67, Asn95, Arg96, Glu97, Gly202, Val214, Tyr216, Ile217, Val263, Lys292, Phe293

4. Discussion

The phenylethanoid glycosides are primarily found in the Verbenaceae, Plantaginaceae, Orobanchaceae, Rosaceae, and Scrophulariaceae families. These families have multiple traditional uses but have not been studied much. These species are prevalent in the south-east asian countries. The decoction from these species are traditionally used for its antimicrobial, antidiabetic, neuroprotective, cardioprotective, and wound-healing effects [5,9]. It is also used in hair care, reduces pain during parturition, and is a part of regular diet for many tribal people [9].

The molecular docking study of the phenylethanoid glycosides with the GSK3-β was successfully conducted. The minimum binding energy was found between −10.2 and −7.3 kcal/mol, showing a good binding affinity between the ligands and the protein. The binding energy of crenatoside was found to be −10.2 kcal/mol, which was the least among all the other ligands. The estimated inhibition constant was in the range of 0.032 to 4.397, suggesting that the ligands are potent inhibitors of GSK3-β even at the lowest concentrations. Therefore, the phenylethanoid glycosides will be the group of choice for faster healing of chronic wounds in the case of diabetes mellitus. However, further animal studies are required to confirm the activity of these phenylethanoid glycosides.

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