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Impact of Fenugreek Oil on Sitagliptin's Pharmacokinetic and Pharmacodynamic Profile: A Computational Docking Study to Correlate Blood Glucose Level

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Abstract

Sitagliptin, a DPP-4 inhibitor, performs by blocking the enzyme dipeptidyl peptidase-4 (DPP-4), which usually degrades incretin hormones that regulate blood glucose level. By inhibiting DPP-4, Sitagliptin enhances incretin levels, promoting insulin release and reducing liver glucose production. This study aimed to investigate the pharmacokinetic and pharmacodynamic interaction in between Sitagliptin, and fenugreek oil. The pharmacokinetic interactions were investigated with molecular docking studies and the results revealed that the absorption of Sitagliptin may be more in presence of fenugreek oil due to high binding energy of linoleic acid, linolenic acid and oleic acid present in fenugreek oil with P-Glycoprotein (P-gp). The albumin binding capacity is less from the fatty acids of fenugreek oil compared to Sitagliptin. The metabolism of Sitagliptin caused by CYP3A4 is less in presence of fenugreek oil as the docking score is more with the components of fenugreek oil. This molecular docking study predicts an improvement of oral bioavailability of Sitagliptin in presence of fenugreek oil due to increased absorption and reduced metabolism. Pharmacodynamic studies were carried out by observing the blood glucose level in Streptozotacin induced diabetic rats. Higher percentage reduction in blood glucose was observed from the group treated with Sitagliptin and fenugreek oil compared to test group treated with Sitagliptin. Thus molecular docking studies are correlated with in-vivo experimental blood glucose data.

Keywords: Sitagliptin, Fenugreek Oil, P-glycoprotein, Linoleic Acid, Linolenic Acid, Oleic Acid.

Introduction

Sitagliptin is primarily served in the management of type 2 diabetes mellitus (T2DM) (1) and it is a dipeptidyl peptidase-4 inhibitor. It helps to controls blood glucose levels by amplifying insulin secretion and lowering glucagon release (2). Sitagliptin inhibits the DPP-4 enzyme, which is responsible for breaking down incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (3). Incretins regulate blood glucose through stimulating insulin secretion in response to meals and inhibiting glucagon secretion, reducing hepatic glucose production, particularly during fasting or between meals (4). Through inhibiting DPP-4, Sitagliptin increases the levels and extend the duration of the action of incretin hormones, enhancing glucose control without triggering excessive insulin release (5). Sitagliptin is quickly absorbed after oral administration with 87% of bioavailability and it is a substrate for P-gp, which may affect its absorption and distribution (6). It undergoes minimal metabolism, accompanied by 79% of the dose evacuated unchanged in urine via active tubular secretion, and 13%

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metabolized primarily by CYP3A4 and minimally by CYP2C8. Sitagliptin has a moderate volume of distribution (~198 L), low plasma protein binding (~38%) and an elimination half-life of 12.4 hours, with13% excreted in feces, mainly unchanged (7).

Fenugreek seeds were commonly used in traditional medicine for managing diabetes due to their potential to regulate blood glucose levels (8). Building on this historical use, recent research has explored the synergistic effects of combining fenugreek oil with modern diabetes treatments, such as Sitagliptin. Fenugreek oil is an essential oil withdrawn from the seeds of the fenugreek. Fenugreek oil contains various bioactive compounds, including saponins, alkaloids, flavonoids and essential fatty acids like linoleic acid (LA), linolenic acid (LLA) and oleic acid (OA) (9). Fenugreek oil is used as anti-inflammatory, anti-diabetic, antioxidant and antibacterial activity (10). Diosgenin aids in pancreatic β-cell regeneration, stimulating insulin secretion, while 4-Hydroxyisoleucine, an amino acid derivative, directly enhances insulin release (11). Saponins improve insulin sensitivity by facilitating GLUT-4-mediated glucose uptake. Additionally, alkaloids in fenugreek oil inhibit α-glucosidase, slowing carbohydrate breakdown and reducing glucose absorption. Some studies also suggest its role as a GLP-1 modulator, AMPK activator and DPP-IV inhibitor, further contributing to blood glucose regulation (12).

Fenugreek oil when used alongside Sitagliptin, a medication that helps control blood glucose levels by inhibiting the enzyme DPP-4, the combination may offer improved blood glucose control. This combination could provide a more effective, holistic approach to diabetes management, leveraging both traditional and modern therapeutic strategies.

Drug interaction occurs when one drug affects the activity or concentration of another, potentially altering its effectiveness or causing harmful side effects. Pharmacokinetic drug interactions occur when one drug influences the absorption, distribution, metabolism or excretion of another, thereby affecting its concentration and therapeutic effect (13).

Molecular docking is a technique of computation used to forecast the preferred orientation and binding affinity of a ligand (such as a small molecule or drug) to a target protein (14). This process involves imitating the interaction between the ligand and the protein's active site to identify the most likely binding pose and estimate the strength of the interaction (15). This method is broadly used in drug discovery to screen potential compounds for biological activity, optimize lead compounds, and gain insights into molecular recognition mechanisms.

This study explored the pharmacokinetic and pharmacodynamic properties of Sitagliptin in combination with fenugreek oil through molecular docking and *in-vivo* studies. Molecular docking was carried out to examine the interactions between Sitagliptin and the major fatty acids present in fenugreek oil (LA, LLA, OA) with key proteins, including P-glycoprotein (P-gp) (6QEX), Serum Albumin (1AO6) and CYP3A4 (5A1R). The objective was to evaluate their binding affinities and potential effects on absorption, distribution and metabolism providing insights into pharmacokinetics and possible drug interactions. Additionally, *in-vivo* studies were conducted to assess the enhanced anti-diabetic activity of Sitagliptin when combined with fenugreek oil in Wistar rats.

Materials and Methods

Molecular docking studies were conducted by using docking tools like Chimera - *v* 1.18, AutoDock Vina - *v* 1.1.2 and Biovia Discovery Studio - *v* 2024 Client. Sitagliptin gifted from Tetanus India Pvt Ltd, Hyderabad fenugreek oil from Dève Herbes, New Delhi and Streptozotacin from Himedia, Thane.

Retrieving of binding energies and 2D structures of Sitagliptin with P-gp (6QEX), Serum Albumin (1AO6) and CYP3A4 (5A1R)

The P-glycoprotein structure with PDB ID: 6QEX was downloaded from the RCSB Protein Data Bank in PDB format, and

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the canonical SMILES of Sitagliptin was retrieved from the PubChem database (16, 17). Next, the P-gp receptor structure and Sitagliptin ligand were imported into Chimera software for preparation. Structure minimization was performed by deleting extra chains and non-standard amino acids from the receptor. The SMILES format of Sitagliptin was converted into a 3D structure, and the grid box was set up. The processed receptor structure was then saved as receptor.pdb. In AutoDock tool, further preparation steps were carried out. Water molecules were removed from the receptor, hydrogen atoms were added, and Kollman charges were assigned. The receptor structure was saved as *full_receptor.pdbqt*. The ligand was also prepared, saved as *ligand.pdbqt*, and the receptor was saved as *receptor.pdbqt* (18). Following this, grid parameter file (*a.gpf*) and docking parameter file (*a.dpf*) were generated using AutoDock tools. The command prompt was used to generate *a.glg* and *a.dlg* files from *a.gpf* and *a.dpf* (19). Docking analysis was performed in AutoDock, where the docking log file (*a.dlg*) and *receptor.pdbqt* were analyzed to determine the binding energy of Sitagliptin with P-gp (20). Finally, Biovia Discovery Studio software was used to visualize the results, generating a 2D interaction diagram of Sitagliptin with P-gp, highlighting key binding interactions (21). The same procedure was repeated for Sitagliptin with serum albumin (1AO6) and CYP3A4 (5A1R).

Retrieving of binding energies and 2D structures of LA, LLA, and OA of Fenugreek oil with P-gp (6QEX)

The binding energies and 2D structures of LA, LLA and OA from fenugreek oil with P-glycoprotein (6QEX) were retrieved using the same procedure mentioned above.

Retrieving of binding energies and 2D structures of LA, LLA, and OA of Fenugreek oil with Serum Albumin (1AO6)

The binding energies and 2D structures of LA, LLA, and OA from fenugreek oil with serum albumin (PDB: 1AO6) were retrieved using the same procedure mentioned above.

Retrieving of binding energies and 2D structures of LA, LLA, and OA of Fenugreek oil with CYP3A4 (5A1R)

The binding energies and 2D structures of LA, LLA, and OA from fenugreek oil with CYP3A4 (PDB: 5A1R) were retrieved using the same procedure mentioned above.

Anti-diabetic activity protocol

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Bapatla College of Pharmacy, with approval No. IAEC/ XVII/03/BCOP/2024. Upon approval, the rats were divided into three groups, each containing 6 animals. Aqueous solution of Sitagliptin 1% w/v was prepared. The solution containing equivalent to 0.8 mg of Sitagliptin (5.12 mg/kg) was used as standard drug. The same amount of Sitagliptin was dissolved in fenugreek oil and the resulting oily solution was served as test solution (22). The first group served as the positive control, the second group was treated with the standard drug and the third group received the test formulation. Prior to treatment, all groups were administered Streptozotocin (65 mg/kg, i.p.) to induce diabetes. Blood glucose levels were measured on the 4th day using an Accu-check glucometer (23). Blood glucose value above 120 mg/dl confirmed the onset of diabetes. Treatment was followed to each group daily for a period of 28 days. Fasting blood glucose values were noted on the 7th, 14th, 21st, and 28th days (24). Percentage blood glucose reduction values were calculated and treated statistically with one way ANOVA.

Results and Discussion

Table 1 presents the binding energy values and 2D conformations of Sitagliptin docked with P-gp (6QEX), serum albumin (1AO6) and CYP3A4 (5A1R). Molecular docking studies were carried out to evaluate the interaction of Sitagliptin with these targets, and the recorded binding energy values were -5.61 kcal/mol for P-gp (6QEX), -6.34 kcal/mol for serum albumin (1AO6) and

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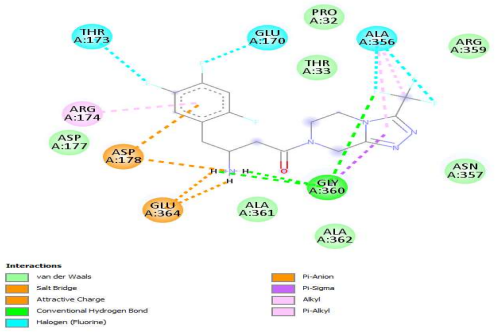
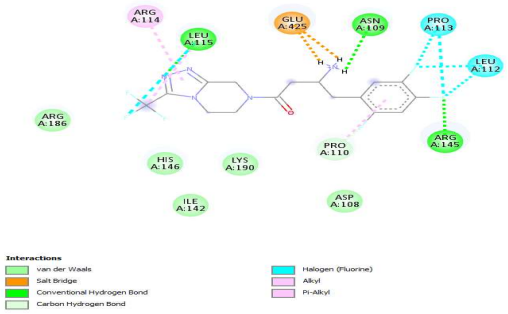
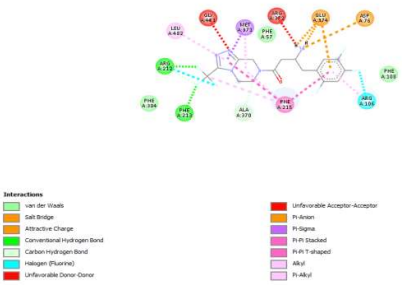
-6.54 kcal/mol for CYP3A4 (5A1R), along with their corresponding 2D conformations.

Table 2 presents the binding energy values and 2D conformations of LA, LLA and OA docked with P-gp (6QEX). Molecular docking studies were conducted to evaluate the interaction of LA, LLA, and OA acid with P-gp (6QEX) and the binding energy values (kcal/mol) were recorded as -5.93, -6.49 and -5.28 respectively, illustrating their 2D conformations and interaction strengths.

Table 3 presents the binding energy values and 2D conformations of LA, LLA, and OA docked with serum albumin (1AO6).

Table 1: Retrieval of binding energies and 2D conformation of Sitagliptin with P-gp (6QEX), Serum Albumin (1AO6) and CYP3A4 (5A1R)

S. No.	Drug	Binding Energy	2D Conformation
1.	Sitagliptin+ Pgp	-5.61	
2.	Sitagliptin+ Serum Albumin	-6.34	
3.	Sitagliptin+ CYP3A4	-6.54	

Correlate Blood Glucose Level

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Table 2: Retrieval of binding energies and 2D conformation of LA, LLA and OA docked with P-gp (6QEX)

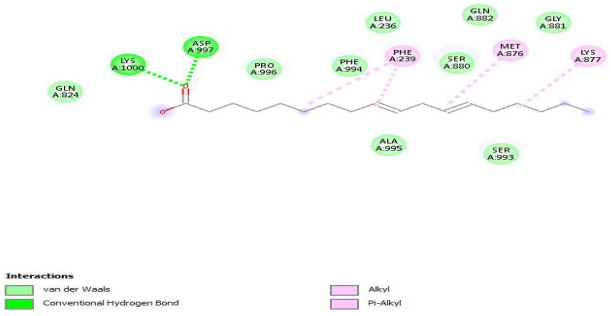
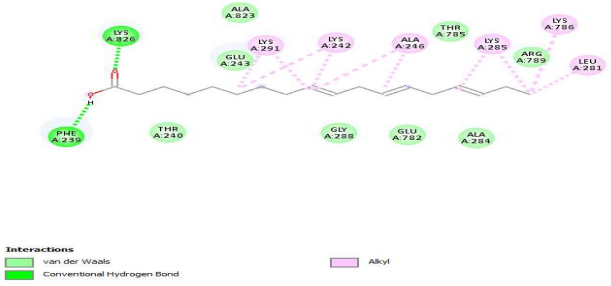
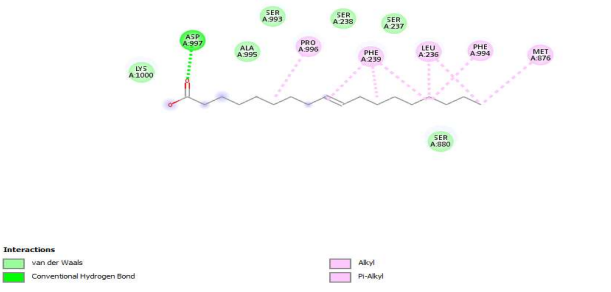
S. No.	Fatty Acid	Binding Energy	2D Conformation
1.	Linoleic Acid	-5.93	
2.	Linolenic Acid	-6.49	
3.	Oleic Acid	-5.28	

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Table 3: Retrieval of binding energies and 2D conformation of LA, LLA and OA docked with Serum Albumin (1AO6)

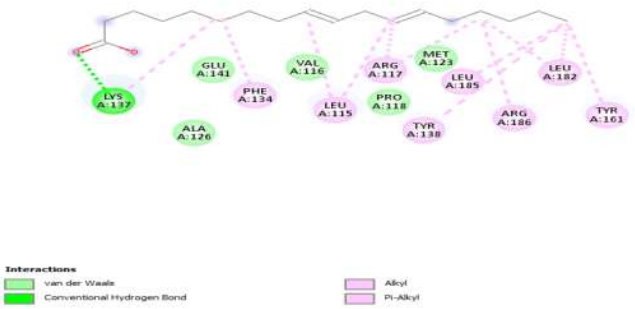
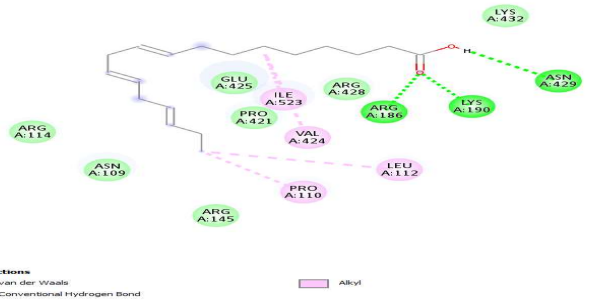
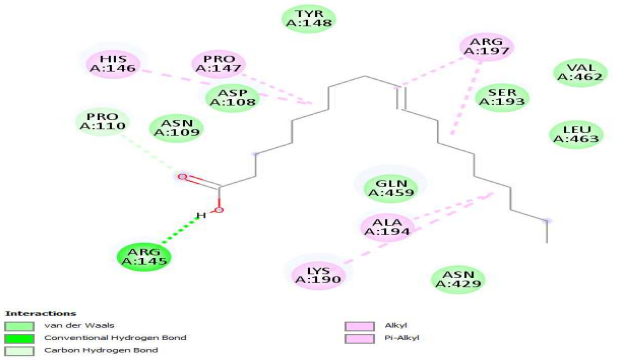
S. No.	Fatty Acid	Binding Energy	2D Conformation
1.	Linoleic Acid	-4.8	
2.	Linolenic Acid	-5.37	
3.	Oleic Acid	-3.96	

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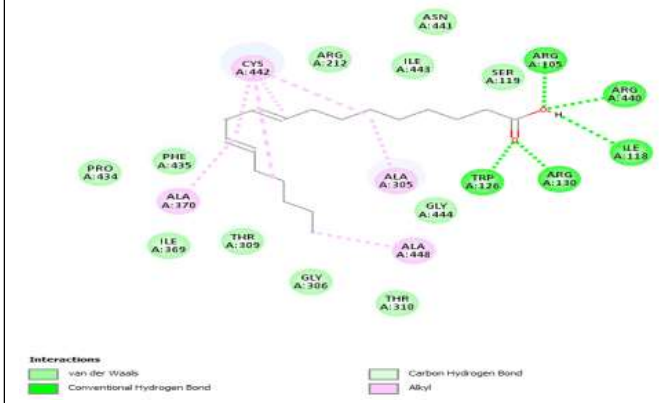
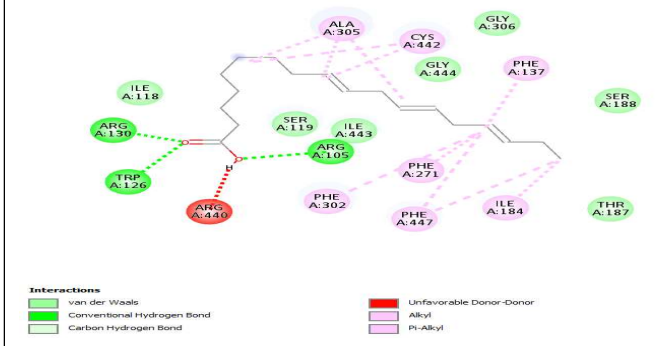
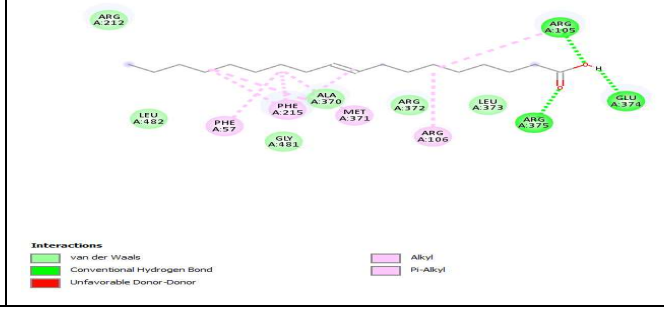
Table 4: Binding energies and 2D conformation of LA, LLA and OA docked with CYP3A4 (5A1R)

S. No.	Fatty Acid	Binding Energy	2D Conformation
1.	Linoleic Acid	-7.5	
2.	Linolenic Acid	-8.6	
3.	Oleic acid	-6.02	

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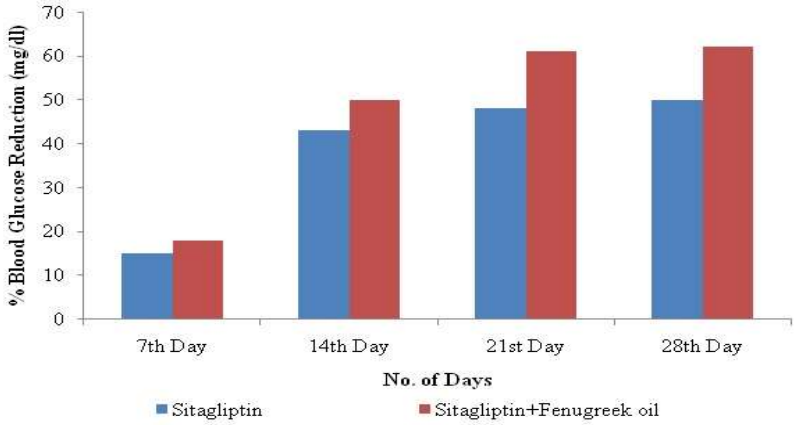

Figure 1: A graphical representation of the % blood glucose reduction in rats over days

Molecular docking studies were carried out to evaluate the interaction of LA, LLA, and OA with serum albumin (1AO6) and the binding energy values (kcal/mol) were recorded as -4.8, -5.37 and -3.96 respectively, illustrating their 2D conformations and interaction strengths.

Table 4 presents the binding energy values and 2D conformations of LA, LLA, and OA docked with CYP3A4 (5A1R). Molecular docking studies were performed to evaluate the interaction of LA, LLA and OA with CYP3A4 (5A1R) and the binding energy values (kcal/mol) were recorded as -7.5, -8.6 and -6.02 respectively, illustrating their 2D conformations and interaction strengths.

The molecular docking results highlight the comparative binding affinities of Sitagliptin and key components of fenugreek oil (LA, LLA, and OA) with P-gp (6QEX), serum albumin (1AO6) and CYP3A4 (5A1R). For P-gp (6QEX), Sitagliptin exhibited a binding energy of -5.61 kcal/mol, while LA (-5.93 kcal/mol), LLA (-6.49 kcal/mol) and OA (-5.28 kcal/mol) demonstrated stronger or comparable affinities. This suggests that fenugreek oil components interact more effectively with P-gp than Sitagliptin, potentially affecting drug efflux mechanisms. Similarly, for CYP3A4 (5A1R), Sitagliptin had a binding energy of -6.54 kcal/mol, whereas LA (-7.5 kcal/mol), LLA (-8.6 kcal/mol) and OA (-6.02 kcal/mol) showed higher affinities. These results indicate that fenugreek oil components bind more strongly to CYP3A4, an enzyme involved in drug metabolism, suggesting a potential impact on Sitagliptin's bioavailability when co-administered. However, in the case of serum albumin (1AO6), Sitagliptin displayed a stronger binding affinity (-6.34 kcal/mol) compared to LA (-4.8 kcal/mol), LLA (-5.37 kcal/mol) and OA (-3.96 kcal/mol). This suggests that Sitagliptin has a higher tendency to bind with serum albumin, which may influence its distribution and transport in the bloodstream.

In-vivo Studies

Figure 1 illustrate that the percentage reduction of blood glucose levels observed in rats. This study clearly demonstrates that the reduction of blood glucose was significantly greater in rats treated with the test group (Sitagliptin and fenugreek oil) compared to those treated with the standard group (Sitagliptin alone) over 7^{th}, 15^{th}, 21^{st} and 28^{th} days.

The combination of Sitagliptin and fenugreek oil resulted in a higher percentage inhibition of blood glucose levels compared to Sitagliptin alone over 28 days. On day 7^{th}, the test group exhibited an 18% inhibition, slightly exceeding the 15% observed in the

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standard group. By day 28th, the test group achieved a 61% inhibition, compared to 50% in the standard group. These results demonstrate that fenugreek oil enhances the anti-hyperglycemic effect of Sitagliptin, leading to improved glycemic control.

Conclusion

This study demonstrated that fenugreek oil altered the pharmacokinetic and pharmacodynamic parameters of Sitagliptin, leading to improved oral bioavailability and anti-hyperglycemic activity. Molecular docking results suggested increased absorption and reduced metabolism of Sitagliptin in the presence of fenugreek oil. *In-vivo* studies in diabetic rats confirmed anti-hyperglycemic activity from the test product improved. The correlation between molecular docking and experimental data supports the potential alternative anti-diabetic therapy. Overall, this study successfully developed a novel formulation with enhanced anti-hyperglycemic activity.

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Conflicts of Interest

No conflicts of interest.

References

1. Kazi, M., Alqahtani, A., Ahmad, A., Noman, O.M., Aldughaim, M.S., Alqahtani, A.S. and Alanazi, F.K. (2021). Development and Optimization of Sitagliptin and Dapagliflozin Loaded Oral Self-nano Emulsifying Formulation Against Type 2 Diabetes Mellitus. *Drug Delivery,* 28(1), 100-114.
2. Yin, R., Xu, Y., Wang, X., Yang, L. and Zhao, D. (2022). Role of Dipeptidyl Peptidase 4 Inhibitors in Antidiabetic Treatment. *Molecules*, 27(10), 3055.
3. Saini, K., Sharma, S. and Khan, Y. (2023). DPP-4 inhibitors for treating T2DM - hype or hope? An analysis based on the current literature. *Frontiers in Molecular Biosciences,* 10, 1130625.
4. Nasr, N.E. and Sadek, K.M. (2022). Role and Mechanism(s) of Incretin-dependent Therapies for Treating Diabetes Mellitus. *Environmental Science and Pollution Research*, 29(13), 18408-18422.
5. Boer, G.A. and Holst, J.J. (2020). Incretin Hormones and Type 2 Diabetes-Mechanistic Insights and Therapeutic Approaches. *Biology*, 9(12), 473.
6. Fares, M., Al-Ani, I.S., Hailat, M., Khaleel, A., Collier, P., Al-Ani, A.S. and Dayyih, W.A. (2021). Evaluation the Impact of Grapefruit Juice on Sitagliptin Pharmacokinetics in Healthy Rats. *International Journal of Pharmaceutical Research,* 13(2), 2145-2153.
7. Gu, N., Park, S.I., Chung, H., Jin, X., Lee, S. and Kim, T.E. (2020). Possibility of Pharmacokinetic Drug Interaction Between a DPP-4 Inhibitor and a SGLT2 Inhibitor. *Translational and Clinical Pharmacology*, 28(1), 17.
8. Saini, T., Sharma, S., Dash, K.K., Sandhu, R., Dadwal, V., Shams, R. and Pandey, V.K. (2025). Role of Bioactive Phytochemicals in Plant Seeds and Leaves for Diabetes Control and Prevention: A Comprehensive Review. *Phytochemistry Reviews*, 1-26.
9. Husen, A. (Ed.). (2023). *Antidiabetic Medicinal Plants and Herbal Treatments,* CRC Press.
10. Almuzaini, N.A.M., Sulieman, A.M.E., Alanazi, N.A., Badraoui, R. and Abdallah, E.M. (2024). Mass Spectrometric Based Metabolomics of the Saudi Cultivar of Fenugreek (*Trigonella foenum-graecum* L.): A Combined GC-MS, Antimicrobial and Computational Approach. *Pharmaceuticals*, 17(12), 1733.
11. Baset, M., Ali, T., Elshamy, H., El Sadek, A., Sami, D., Badawy, M., Abou-Zekry, S., Heiba, H., Saadeldin, M. and Abdellatif, A. (2020). Anti-diabetic Effects of Fenugreek (Trigonella foenum-graecum): A Comparison between Oral and Intraperitoneal Administration - An Animal Study. *International Journal of Functional Nutrition*, 1(1), 1-9.

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10.5530/ctbp.2025.2s.8

12. Sarker, D.K., Ray, P., Dutta, A.K., Rouf, R. and Uddin, S.J. (2024). Antidiabetic Potential of Fenugreek (Trigonella foenum-graecum): A Magic Herb for Diabetes Mellitus. *Food Science & Nutrition*, 12(10), 7108-7136.

13. Moon, S.J., Yu, K.S. and Kim, M.G. (2020). An Assessment of Pharmacokinetic Interaction Between Lobeglitazone and Sitagliptin After Multiple Oral Administrations in Healthy Men. *Clinical Therapeutics*, 42(6), 1047–1057.

14. Stanzione, F., Giangreco, I. and Cole, J.C. (2021). Use of Molecular Docking Computational Tools in Drug Discovery. *In Progress in Medicinal Chemistry*, 60, 273-343.

15. Wei, Y., Palazzolo, L., Ben Mariem, O., Bianchi, D., Laurenzi, T., Guerrini, U. and Eberini, I. (2024). Investigation of In Silico Studies for Cytochrome P450 Isoforms Specificity. *Computational and Structural Biotechnology Journal*, 23, 3090-3103.

16. Teng, Y.N., Huang, B.H., Huang, S.Y., Wu, I.T., Wu, T.S., Lee, T.E. and Hung, C.C. (2021). Cinnamophilin Overcomes Cancer Multi-drug Resistance via Allosterically Modulating Human P-glycoprotein on Both Drug Binding Sites and ATPase Binding Sites. *Biomedicine & Pharmacotherapy*, 144, 112379.

17. Ghorbanzadeh, V., Aljaf, K.A.H., Wasman, H.M., Pirzeh, L., Azimi, S. and Dariushnejad, H. (2022). Carvacrol Enhance Apoptotic Effect of 5-FU on MCF-7 Cell Line via inhibiting P-glycoprotein: An In-silco and *In-vitro* Study. *Drug Research*, 72(04), 203-208.

18. Mouithys-Mickalad, A., Etsè, K.S., Franck, T., Ceusters, J., Niesten, A., Graide, H., Deby-Dupont, G., Sandersen, C. and Serteyn, D. (2024). Free Radical Inhibition Using a Water-Soluble Curcumin Complex, NDS27: Mechanism Study Using EPR, Chemiluminescence and Docking. *Antioxidants*, 13(1), 80.

19. Wei, Y., Palazzolo, L., Ben Mariem, O., Bianchi, D., Laurenzi, T., Guerrini, U. and Eberini, I. (2024). Investigation of In Silico Studies for Cytochrome P450 Isoforms Specificity. *Computational and Structural Biotechnology*, 23, 3090-3103.

20. Ranjbar, S., Lashkarian, F.F., Khoshneviszadeh, M., Moosavi, F., Sakhteman, A., Zargari, F., Saso, L., Firuzi, O. and Edraki, N. (2023). 5-Oxohexahydroquinolines bearing 4-pyridyl methyl carboxylate as P-glycoprotein inhibitors and multidrug resistance reversal agents in cancer cells. *Journal of Molecular Structure*, 1285, 135427.

21. Umesh, H.R., Ramesh, K.V. and Devaraju, K.S. (2020). Molecular Docking Studies of Phytochemicals against Trehalose–6–phosphate Phosphatases of Pathogenic Microbes. *Beni-Suef University Journal of Basic and Applied Sciences*, 9(1), 5.

22. Majumdar, J., Chatterjee, A., Chakraborty, S.B., Chakraborty, P. and Sarkar, S. (2024). Comparative Efficacy of Four Potent Bioactive Molecules of Fenugreek Seed in Holistic Management of Type 1 Diabetes. *Advances in Traditional Medicine*, 1-4.

23. Limaki, H.K. *In-vivo* and *In-vitro* Study of Mechanism of Action of 4 hydroxyisoleucine as an Amino Acid Derived from Fenugreek Seed with Anti-diabetic and Properties (Doctoral Dissertation, London Metropolitan University).

24. Zayed, E.A., Ain Shoka, A.A., El Shazly, K.A., El-Mosallamy, A.E.M.K., Zayed, A.A. and Abd El Latif, H.A. (2021). A 4-Week Period of Administration of Fenugreek Oil or Metformin Improve Cardiac and Renal Complications in Metabolic Syndrome-Induced Rats. *Asian Journal of Cardiology Research*, 4(1), 53-64.