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INVITRO DRUG INTERACTION STUDY OF ALOGLIPTIN BESYLATE WITH TELMISARTAN ANDAZILSARTAN BY EQUILIBRIUM DIALYSIS USING UVSPECTROSCOPIC METHOD

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ABSTRACT

Introduction : This study mainly aimto investigate *In vitro* protein binding and drug displacement study of Alogliptin Besylate (ALO) with the selected Angiotensin II Receptor Blockers (TEL and AZIL) using equilibrium dialysis by UV spectroscopic method.

Method: To study the protein binding and drug displacement property of ALO with selected sartans by using the equilibrium dialysis sac method. The effect of TEL and AZIL on *in vitro* protein binding of ALO was studied at different ratios (BSA: ALO:TEL/AZIL, 1:1:1,1:1:2,1:1:3,1:1:4). And this procedure is repeated vice versa by adding Alogliptin into increasing concentration to get a final ratio (BSA:TEL/AZIL:ALO, 1:1:1,1:1:2,1:1:3,1:1:4)for study the effect of ALO on *in vitro* protein binding of TEL and AZIL. Then responses of free drug concentrations were measured. The possibility of protein-drug binding interaction is confirmed by using Molecular Docking Studies.

Result: The specific site of protein binding of the drugs was confirmed by molecular docking studies. The percentage protein binding of ALO, TEL and AZIL was found to be ≤ 21.4%, ≤95.5%, and ≤93.2% respectively. The displacement property of each drug was studied.

Conclusion: There are no drug-drug interactions are occurring between the ALO and selected sartans but as compared to AZIL and TEL with ALO, AZIL is the more preferable choice for alogliptin when they use together.

Keywords: Dipeptidyl peptidase-4 inhibitor, Angiotensin-2 receptor blocker, Bovine serum albumin, Human serum albumin.

INTRODUCTION:

Drug interactions commonly occur when two or more drugs are taken together by a person. Some drug interactions should not be avoided or reversed because that will lead to serious harm to the patients. Drug-drug interactions also play important role in extending the protein binding of drugs and altering their therapeutic effects. It is also affecting toxicity and drug stability during the therapeutic process. So, it is very important to assess the type and extent of binding interaction with protein and drug that will occur.[1,2]The plasma protein binding is an important pharmacokinetic parameter of drugs. If there are any alterations in physiological conditions that will cause the changes in the pharmacokinetic and pharmacodynamic profile of drugs.Most of the drugs especially newly marketed having multiple pharmacological effects on the body. when the patients are taken multiple prescriptions increase the risk of drug-drug interactions. So, it is very important to study and find out such interactions in the body.[3,4]Diabetes and hypertension are the most commonly occurring lifestyle diseases nowadays. There are lots of people having

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both diabetes and hypertension. So, in such cases, they will take the medicine for both diseases. And some cases the hypertensive drugs are prescribed with diabetic medication to control the blood pressure level in diabetic patients to reduce the risk of cardiovascular diseases. While taking both medication for BP and diabetes increases the chances of drug-drug interaction in those patients.[5,6]

In the current study, we are mainly focused on to *in vitro* displacement interaction study of the drug Dipeptidyl peptidase-4(DPP-4) inhibitor(DPP-4) [alogliptin (ALO)] with Angiotensin-2 receptor blockers (ARBs)[telmisartan (TEL) and azilsartan (AZIL)]. The ALO is a newer class of oral antidiabetic agent coming under the class of DPP-4 inhibitor. Which is prescribed in the patient when they are resistant to other oral antidiabetics such as metformin, sulfonyl urease, biguanides, etc. The telmisartan and azilsartan are the ARBs coming under the class of antihypertensives and concomitant treatment with ARBs in combination with DPP-4 inhibitors is increasing. [7,8]

In this study, we are going to check whether there is any interaction occurs when these two drugs are taken together by using *invitro* analytical method. And also did molecular docking studies to predict the possibility of protein binding with these drugs by using docking software.The objective of the study is to investigate and understand the *invitro* displacement interaction of drug ALO with selected ARBs and find out there is any drug-drug interactions are occurred or not and also predict the possibility of protein binding of drugs by using molecular docking studies. Bovine serum albumin (BSA) is used for this study instead of human serum albumin (HSA) due to its structural similarity, low cost, and easy availability of BSA. The *invitro* protein binding of alogliptin with selected ARBs has been conducted by the equilibrium dialysis method.

MATERIALS AND METHODS

Materials

ALO was provided by Glenmark Pvt. Ltd (Maharashtra, India), TEL and AZIL were provided by MSN (Hyderabad, India). BSA was procured from Himedia Laboratories Pvt. Ltd.(Mumbai, India), Dialysis sac (21 mm diameter, 30 cm length) (Mumbai), Potassium dihydrogen phosphate (AR grade) was supplied by S.D Fine chemicals Ltd. (Mumbai, India) and HPLC grade water (Millipore Milli-Q Purification System).

Instrumentation

The UV-Vis spectra were recorded on a SHIMADZU UV-1700 Pharmaspec UV-Vis spectrometer. All weighing was done on a SHIMADZU AUX220-Analytical balance.

Preparation of reagents

Buffer solution pH 7.4

A 25 ml of potassium dihydrogen solution was added 19.55 ml of sodium hydroxide solution and volume made up to 100 ml with distilled water.

Potassium dihydrogen solution

A 2.72 gm of potassium dihydrogen phosphate was dissolved and volume was made up to 100 ml with distilled water.

0.2 N sodium hydroxide solution

A 4 gm of sodium hydroxide was dissolved and volume was made up to 100 ml with distilled water.

Bovine serum albumin (1×10^{-5})

A 0.165 gm of BSA was dissolved and made up to 25 ml with buffer solution.

Preparation of standard curve

The estimation of drug concentrations was done at different concentrations of drug solutions prepared with buffer solution (pH 7.4), absorbance was taken at their respective wavelength and plot standard curve for three drugs.

Equilibrium dialysis method [9]

Study of protein binding of individual drugs

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The protein binding of all the drugs is determined by the equilibrium dialysis method. For this, 25 ml of $1x10^{-5}$ M concentrations of all the drug solutions and BSA is prepared. The 25 ml of $1x10^{-5}$ M BSA solution is taken in a glass tube attached to a semipermeable membrane. The dialysis membranes are activated previously by immersing them in warm water for 30 min. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing a fixed concentration of drug solutions ($1x10^{-5}$ M). Immediately at zero-time, 1 ml of the solution is pipetted out from the beaker and it is replaced with 1 ml of phosphate buffer of pH 7.4. Readings were taken at various time intervals by UV spectrophotometer at respective λ_{max} of all the drugs till the absorbance values were constant.

Study of the effect of TEL and AZIL on *in vitro* protein binding of ALO

To study the effect of TEL and AZIL on *in vitro*protein binding of ALO, 25 ml of $1x10^{-5}$ M BSA was taken in the two-glass tube attached with membrane after the activation of the membrane with warm water. Then immerse the BSA containing tubes into beakers containing 25 ml of phosphate buffer with a fixed concentration of ALO, then add the TEL and AZIL into both beakers with increasing concentration to give a final ratio (BSA: ALO: TEL/AZIL, 1:1:1,1:1:2,1:1:3,1:1:4). This system is maintained at room temperature for 6 hrs. After 6hrs 1ml of the solutions were withdrawn from beakers and diluted to 10 ml using phosphate buffer and the responses of free drugs were measured at a specific wavelength using the UV spectroscopic method

***In vitro* interaction of ALO with TEL and AZIL using BSA**

To study the effect of TEL and AZIL on *in vitro*protein binding of ALO, 25 ml of 1×10^{-5} M BSA solution was taken into two glass tubes attached to the semi-permeable membrane which is previously activated. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing fixed concentrations of TEL and AZIL. ALO were added in to increasing concentration to get a final ratio (BSA:TEL/AZIL: ALO, 1:1:1,1:1:2,1:1:3,1:1:4). This is maintained at room temperature for 6 hrs. After 6 h, 1 ml of the solutions were withdrawn from the beakers, diluted to 10 ml using phosphate buffer and the responses of free drugs were measured. The concentrations of the drugs were determined from the calibration graph.

***In silico*Molecular docking studies**[10,11]

Molecular docking is done with selected drugs as ligand and BSA/HSA as the target is performed by using the Auto dock 4.2 and Auto dock tools 1.5.6(ADT) software. Proteins (BSA/HSA) were downloaded from the protein data bank and the structure of drugs was collected from PubMed. The PDB ID of BSA and HSA was 1HA2 and 3V03 respectively. The BIO-via 2020 discovery studio software was utilized to study the best binding mode/conformation of drugs with proteins. Then proteins and drugs are applied to Auto dock software to get the complex was studied. It's suited to investigate and visualize the interaction mechanisms and the possible orientations of the complex.

RESULTS AND DISCUSSION:

Table 1. Calibration data

Drugs	**Linearity range (µg/ml)**	**$\lambda_{max(nm)}$**
ALO	1-10	277
TEL	5-15	295
AZIL	1-10	251

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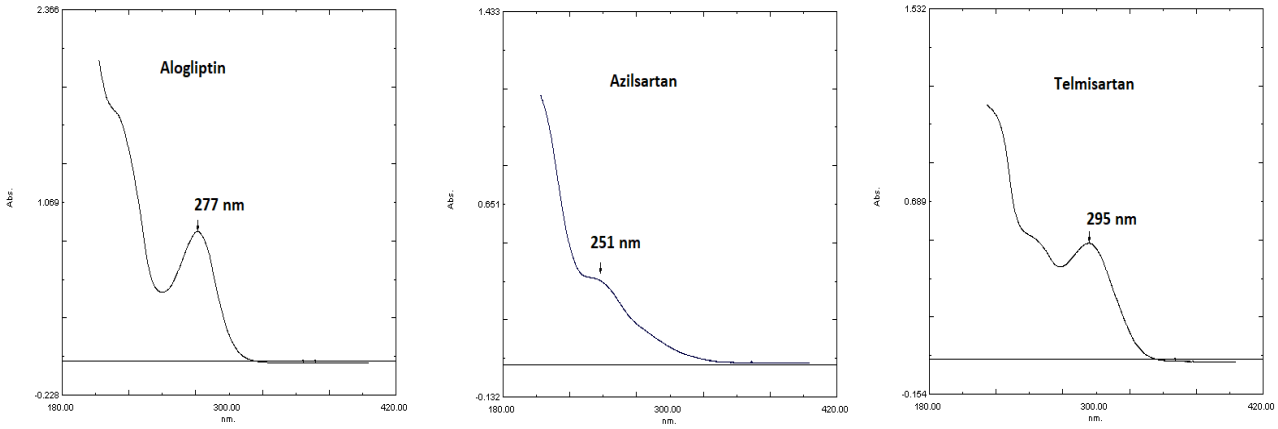

Figure 1 Theλ_{max} of ALO, AZIL and TEL on phosphate buffer (pH 7.4) respectively

Study of Protein Binding on Individual Drugs

The concentration of the drugs was determined from the linearity curve. The percentage protein binding and free drug fraction were calculated. From that, the sartans are shown more than 90% of protein binding and ALO shows only 21.4% of protein binding. (**Table 2)**

Table 2. Protein binding of individual drugs

Drugs	**% of protein binding (n=3)**	**% free drug concentration (n=3)**
ALO	21.40 ±0.0011	78.59 ±0.0012
TEL	95.54 ±0.0013	4.45 ±0.0011
AZIL	93.22 ±0.0012	6.77 ±0.0014

Displacement Interaction Studies

Effect of TEL/AZIL on *invitro* protein binding of ALO

The TEL shows more interaction with ALO as compared to AZIL. The free fraction of ALO is increases from 79.42% to 84.57% while increasing the ratio of TEL with BSA up to 1 to 4. In the case of AZIL on ALO, the free fraction of ALOincreases from 80.05% to 84.33%. Hence, increasing concentration of free drugsindicated that TEL displaced by ALO more as compared with AZIL**(Table 3 and Figure 2).**

Table 3. Effect of TEL/AZIL on *in-vitro* protein binding of ALO

BSA: ALO:TEL/AZIL (n=3)	**% of protein binding of ALO**		**% of free drug concentration of ALO**	
	With TEL	**With AZIL**	**With TEL**	**With AZIL**
1:1:1	20.56	19.95	79.42	80.05
1:1:2	18.86	18.30	81.14	81.70
1:1:3	18.15	17.38	81.85	82.62
1:1:4	15.43	15.67	84.57	84.33

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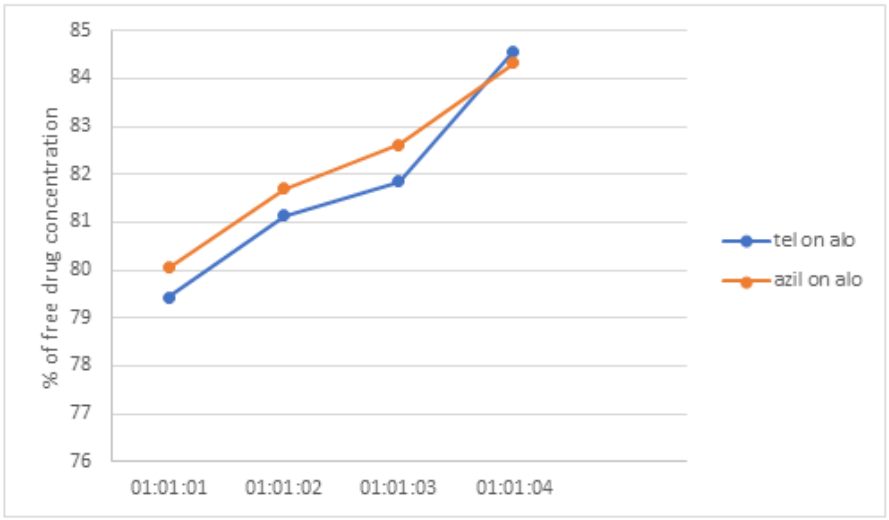

Figure 2 Effect of TEL/AZIL on *in-vitro* protein binding of ALO

Effect of ALO on *in vitro* protein binding of TEL/AZIL

In the reverse experiment, the free drug concentration was found to be more as compared to AZIL. In the presence of an increasing concentration of ALO the free fraction of TEL increases from 4.80% to 6.82% and in the case of AZIL, it was 8.50% to 9.54%. Hence, the ALO displaces TEL more than AZIL**(Table 4 and Figure 3).**

Table4. Effect of ALO on *in vitro* protein binding of TEL/AZIL

BSA: TEL/AZIL: ALO (n=3)	**% of protein binding**		**% of free drug concentration**	
	TEL	**AZIL**	**TEL**	**AZIL**
1:1:1	95.20	91.50	4.80	8.50
1:1:2	94.79	90.86	5.21	9.14
1:1:3	93.18	90.46	6.01	9.54
1:1:4	93.99	90.46	6.82	9.54

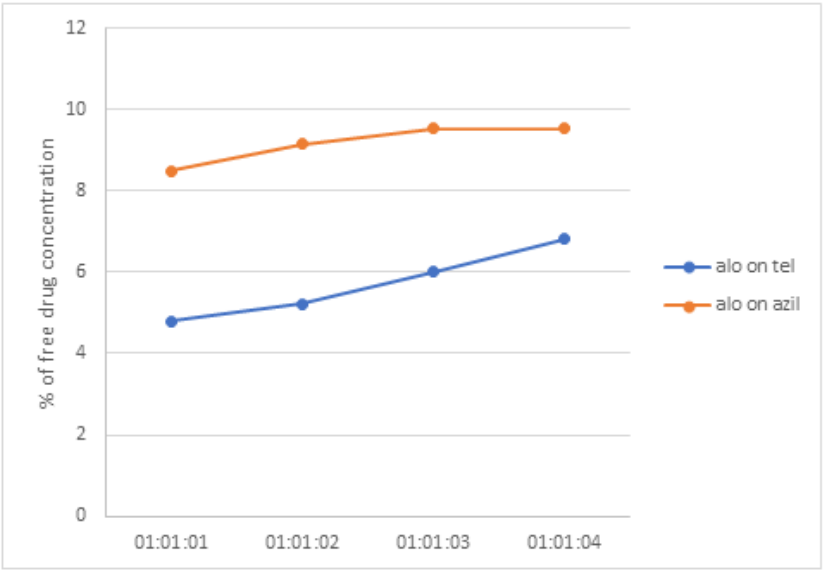

Figure 3. Effect of ALO on *in vitro* protein binding of TEL/AZIL

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Comparison of the effect of antihypertensive drugs (TEL and AZIL) on *in vitro* protein binding of the antidiabetic drug (ALO) carried out **(Figure 2)**, in presence of TEL free fraction of ALO were increased from 79.42% to 84.57% and in case of AZIL, it was increased up to 80.05% to 84.33%. it was 0.87% less than TEL. So, it is evident for among the two hypertensive drugs AZIL is more suitable for concomitant administration of ALO.Then a comparison between the effect of the antidiabetic drug (ALO) on *in vitro* protein binding of antihypertensive drugs (TEL and AZIL) were studied **(Figure 3)**, the free fraction of TEL increases from4.80% to 6.82% and in the case of AZIL, it was 8.50% to 9.54%. it was 0.98% less than TEL. This data is also evidence that the interaction of ALO with AZIL is less as compared to ALO with TEL.

While comparing the data we can conclude the study that AZIL is the more preferred choice for the concomitant administration of ALO as compared with TEL. And also, the data were shows more than 75% of ALO were found as a free drug fraction so less than 25% go for protein binding. And in the case of sartans more than 90% were found as protein-bound. So that will indicate there are no significant interactions are occur the ALO with sartans they are safe to use together.

Molecular docking studies

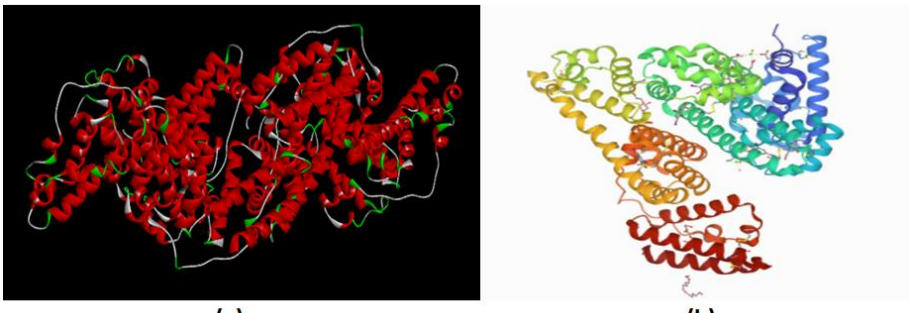
To further determineif binding of protein with drugs for the following three drugs of ALO, TEL and AZIL with BSA and HSA were simulated by molecular docking method implemented in Auto Dock 1.5.6 software. It was then consistently determined that all three drugs bound with site-1 of both HSA and BSA. Based on the docking results all three drugs have binding energy as negative for binding with proteins, which will indicate their binding capacity with proteins is energetically favorable. The ALO is the only drug that shows hydrogen bond mediated interaction with both BSA and HSA. (**Table5** and **Table 6**)

Table 5. The binding energy of drugs on BSA and No.of hydrogen bond present

SL No.	Ligands	Binding energy (ΔG =Kcal/mol)	No. of hydrogen bonds
1	ALO	-8.3	1
2	TEL	-8.9	-
3	AZIL	-8.4	-

Table 6. The binding energy of drugs on HSA and No.of hydrogen bond present

Sl.No	Ligands	Binding energy (ΔG =Kcal/mol)	No. of hydrogen bonds
1	ALO	-7.71	2
2	TEL	-12.43	-
3	AZIL	-8.85	-



(a) (b)

Figure 4a &b. Structure of HSA and BSA

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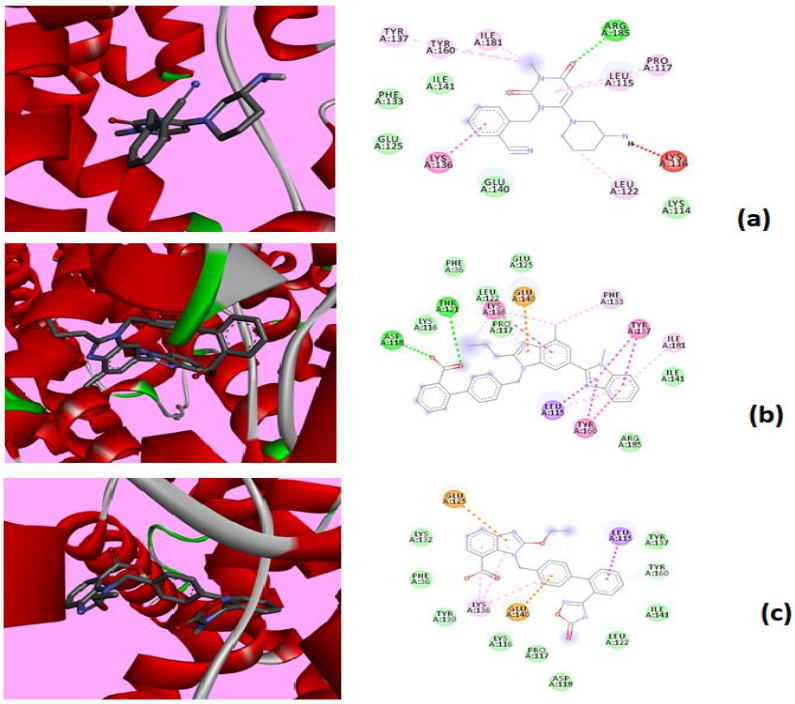

Figure 5a-c Docking results of ALO, TEL and AZIL on BSA respectively

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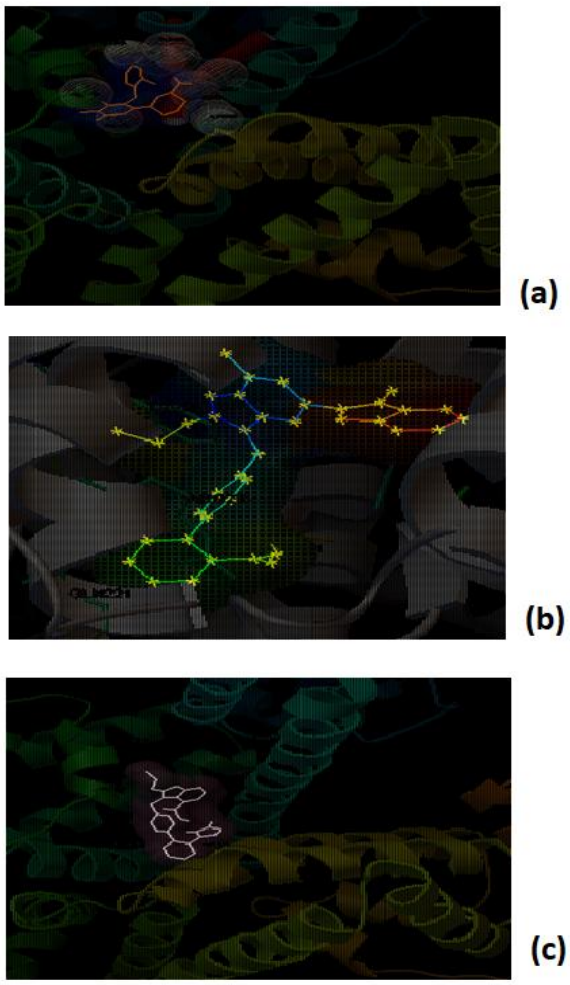

Figure 6a-c. Docking results of ALO, TEL and AZIL on HSA

In the present study, we investigated the drug-drug interaction between the ALO with selected angiotensin II receptor blockers using the *invitro* analytical method. Here ALO is used as antidiabetic and TEL and AZIL are the selected ARBs used as antihypertensives. Before the application of protein displacement study first, we are developing a method for each drug on UV spectroscopy and they are validated according to ICH guidelines Q2A (Supplementary Information). All values of validated parameters were within the limit, so the developed method was found to be accurate, precise and simple.

The binding interaction of each drug with BSA and HSA was confirmed by using molecular docking studies. Which revealed the binding site of each drug with protein BSA and HSA at site -1. Based on the *in vitro* protein binding study of individual drugs, we found that ALO shows only 21.4% of protein binding and TEL and AZIL show 95.5% and 93.3% respectively. Based on the displacement interaction studies we found that the AZIL is the more preferred choice as compared to TEL with ALO. It also indicates that ALO remained free fraction as more than 75% while sartansremain only less than 10% as a free fraction of drug. So, there is no significant interaction are occurred between the ALO and selected sartans (AZIL & TEL), they are safe to use together.

CONCLUSION:

To the best of our knowledge, this study is the first time to investigate the drug-drug interaction between ALO with TEL and AZIL using *invitro* analytical methods by UV spectroscopy. It may conclude that there are no noticeable interactions occur between the ALO and selectedsartans (AZIL & TEL) used together but as compared to AZIL and TEL, AZIL is the more preferred choice with ALO when they use together.

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CONFLICT OF INTEREST:
The authors have no conflicts of interest regarding this investigation**.**

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REFERENCES

1. Lu C, Di L. *In vitro* and in vivo methods to assess pharmacokinetic drug–drug interactions in drug discovery and development. Biopharmaceutics & drug disposition. 2020 Feb;41(1-2):3-1.
2. Treyer A, Ullah M, Parrott N, Molitor B, Fowler S, Artursson P. Impact of intracellular concentrations on metabolic drug-drug interaction studies. The AAPS journal. 2019 Sep;21(5):1-0.
3. Triplitt C. Drug interactions of medications commonly used in diabetes. Diabetes Spectrum. 2006 Oct 1;19(4):202-11.
4. Barré J, Chamouard J, Houin G, Tillement JP. Equilibrium dialysis, ultrafiltration, and ultracentrifugation compared for determining the plasma-protein-binding characteristics of valproic acid. Clinical chemistry. 1985 Jan 1;31(1):60-4.
5. Epstein M, Sowers JR. Diabetes mellitus and hypertension. Hypertension. 1992 May;19(5):403-18.
6. Glovaci D, Fan W, Wong ND. Epidemiology of diabetes mellitus and cardiovascular disease. Current cardiology reports. 2019 Apr;21(4):1-8.
7. Gul S, Basheer S. UV visible spectrophotometric determination, validation and invitro interaction of rabeprazole with chlorazepate. Saudi J. Med. Pharm. Sci. 2017;3(8):813-7.
8. Kang WY, Lee HW, Gwon MR, Cho S, Shim WS, Lee KT, Yang DH, Seong SJ, Yoon YR. A Pharmacokinetic Drug Interaction Between Fimasartan and Linagliptin in Healthy Volunteers. Drug design, development and therapy. 2020;14:2101.
9. Varghese SJ, Johny SK, Paul D, Ravi TK. In vitro interaction study of retinoic acid isomers with telmisartan and amlodipine by equilibrium dialysis method using UV spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2011 Jul 1;79(2):384-7.
10. Bratty MA. Spectroscopic and molecular docking studies for characterizing binding mechanism and conformational changes of human serum albumin upon interaction with Telmisartan. Saudi pharmaceutical journal: SPJ: the official publication of the Saudi Pharmaceutical Society. 2020 May 6;28(6):729-36.
11. Shen GF, Liu TT, Wang Q, Jiang M, Shi JH. Spectroscopic and molecular docking studies of binding interaction of gefitinib, lapatinib and sunitinib with bovine serum albumin (BSA). Journal of Photochemistry and Photobiology B: Biology. 2015 Dec 1;153:380-90.
12. Kurz H, Trunk H, Weitz B. Evaluation of methods to determine protein-binding of drugs. Equilibrium dialysis, ultrafiltration, ultracentrifugation, gel filtration. Arzneimittel-forschung. 1977 Jul 1;27(7):1373-80.
13. Kariv I, Cao H, Oldenburg KR. Development of a high throughput equilibrium dialysis method. Journal of pharmaceutical sciences. 2001 May 1;90(5):580-7.
14. Lin ZJ, Musiano D, Abbot A, Shum L. In vitro plasma protein binding determination of flunarizine using equilibrium dialysis and liquid chromatography–tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis. 2005 Apr 1;37(4):757-62.
15. Plum A, Jensen LB, Kristensen JB. In vitro protein binding of liraglutide in human plasma determined by reiterated stepwise equilibrium dialysis. Journal of pharmaceutical sciences. 2013 Aug 1;102(8):2882-8.
16. Rabbani-Chadegani A, Mollaei H, Sargolzaei J. Investigation of the interaction between berberine and nucleosomes in solution: Spectroscopic and equilibrium dialysis approach. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2017 Feb 15;173:418-24.
17. Rosu F, De Pauw E, Guittat L, Alberti P, Lacroix L, Mailliet P, Riou JF, Mergny JL. Selective interaction of ethidium derivatives with quadruplexes: an equilibrium dialysis and electrospray ionization mass spectrometry analysis. Biochemistry. 2003 Sep 9;42(35):10361-71.
18. van Liempd S, Morrison D, Sysmans L, Nelis P, Mortishire-Smith R. Development and validation of a higher-throughput equilibrium dialysis assay for plasma protein binding. JALA: Journal of the Association for Laboratory Automation. 2011 Feb;16(1):56-67.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

19. Wan H, Rehngren M. High-throughput screening of protein binding by equilibrium dialysis combined with liquid chromatography and mass spectrometry. Journal of Chromatography A. 2006 Jan 13;1102(1-2):125-34.
20. Yasmeen S, Khatun S, Qais FA. Characterization of interactions between cromolyn sodium and bovine serum albumin by spectroscopic, calorimetric and computational methods. Journal of Biomolecular Structure and Dynamics. 2019 Mar 26.