International Journal of Advanced Research in Pharmacy and Education
Volume 2, Issue 2 - 2020, Pg. No. 6-15
Peer Reviewed & Open Access Journal

Research Article

In Silico Toxicity and Efficacy Prediction of a Combination Drug, Namely, "Losartan Potassium and Hydrochlorothiazide"

Rehnuma Tanjin[1], Matrika Saha Roy[2], Tanmoy Debnath[3], Milon Mondal[4], Bidduth Kumar Sarkar[5], Md Murad Hossain[6], Sukalyan Kumar Kundu[7]

[1,2,3,6]Student, [7]Professor, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.
[4]Faculty, Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh.
[5]Scientific Officer, Bangladesh Reference Institute for Chemical Measurements, Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka Bangladesh.

I N F O

Corresponding Author:
Sukalyan Kumar Kundu, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.
E-mail Id:
skkbd415@juniv.edu
Orcid Id:
https://orcid.org/0000-0002-5130-7163

How to cite this article:
Tanjin R, Roy MS, Debnath T et al. In Silico Toxicity and Efficacy Prediction of a Combination Drug, Namely, "Losartan Potassium and Hydrochlorothiazide". *Int J Adv Res Pharm Edu* 2020; 2(2): 6-15.

Date of Submission: 2021-01-11
Date of Acceptance: 2021-02-09

A B S T R A C T

Hypertension, the silent killer is one of the biggest public health concerns. Losartan is the second prescribed anti-hypertensive generic in terms of unit. Here molecular docking approach has been utilized to predict the efficacy and toxicity profile of 'Losartan Potassium and Hydrochlorothiazide' therapy. Docking is inquiring about an appropriate binding site for a ligand that suits energetically and linearly to the protein binding site. Firstly, the ligand was searched in PubChem. Canonical SMILE form was inputted in Protox for toxicity prediction. Swiss Target Prediction was used to find out the target proteins associated with efficacy and toxicity. Proteins are responsible for desired and the undesired effect was downloaded from Protein Data Bank. Undesired ligand complexed was removed by PyMOL Protein and ligand may have unfavourable bond strength, bond length and torsion angle interfering with docking protocol. So Protein and ligand had undergone energy minimization by Swiss PDB Viewer. Lastly, docking of Ligand, namely Losartan and Proteins, namely Endothelin receptor, PPAR gamma and Tyrosine Kinase ABL by PyRx was performed. Discovery Studio was used for visualization of the docking complex. Hydrochlorothiazide is predicted safe as it had shown no toxicity profile in Protox. The three proteins showed a very good vina binding affinity with the ligand. It implies that Losartan causes both desired and undesired effect by binding with the proteins. Proteins responsible for immunotoxicity can form a conventional hydrogen bond, van der Waals interaction, Pi sigma, Pi alkyl and unfavourable donor-donor interaction with Losartan resulting in immunotoxicity and undesired effect. There is no common protein found for Losartan and Hydrochlorothiazide. So there is no chance of interaction for toxicity as well as efficacy. More study should be carried out to acknowledge the drug safer.

Keywords: Hypertension, Losartan Potassium, Hydrochlorothiazide, Molecular Docking, In-silico assessment, Toxicity, Computer-aided-drug-designing

Introduction

It takes almost 15 years for a drug to be marketed from its initial discovery. To be marketed a drug have to pass safety and toxicity screening. But it is impossible to evaluate its exact toxicity based on animal testing or few numbers of the volunteer. So some drawbacks remain. Many drugs exert toxicity after long term use or when used with other drug or dietary supplement. There are lots of evidence that a large number of drugs are recalled or banned from the market after some years of its marketing. Many drugs are available in the market for which a sufficient amount of long term, safety data is available. And some drug comes to the market in the combination dosage form. For those drug long term safety data is difficult to collect. So, they need proper investigation both theoretically and practically. So, nowadays Computer-Aided-Drug-Designing (CADD) approaches are used very widely to increase the efficiency of drug discovery and development. CADD can reduce the time and cost of discovery and development by up to 50%. Molecular docking is a method used to predict the placement of ligands within the active site of their target protein (receptor). Molecular docking gives a favourable estimation of binding strength, binding affinity, bond length, bond angle etc. If a drug proves to be safe in in-silico assessment then it should be permitted for further assessment or animal/human trial. Hypertension is the most commonly encountered disease in this world. Almost 1.13 billion people worldwide have hypertension, resulting in 10.4 million (12.8%) death globally. Losartan is the first anti-hypertensive generic which crossed 100 crores and as a combination added another 86 million crores, Taka. So, in silico research can add another dimension to its safety profile.

Material and Method

The molecular docking approach explores the behaviour of ligand in the binding site of a target protein. Over the last two decades, more than 60 different docking tools and programs have been developed for both academic and commercial use. But this research involves only a few of those. Software used are PubChem, Protox, Protein Data Bank (PDB), FLARE, Swiss Target Prediction, PyRx, PyMOL, Swiss-Pdb Viewer and Discovery Studio.

Initially, 'PubChem' is used to get a molecular formula of the drug (ligand). From PubChem, the ligand/drug had been downloaded in SDF form. Then the Canonical SMILE form is copied to the keyboard for further step. Canonical SMILE formula of Losartan is: CCCCC1=NC(=C(N1CC2=CC=C(C=C2)C3=CC=CC=C3C4=NNN=N4)CO)Cl. Then Protoxhad has been used for toxicity prediction. From the software, it had been found that Losartan may have severe immunotoxicity (probability 0.96), hepatotoxicity and affinity on Aromatase, Estrogen receptor Alpha and Estrogen receptor ligand-binding domain (probability 1.0 or o.99). Then 'Swiss Target Prediction' had been used to see the possible target protein interacting with the ligand. There is almost 70 target protein available for binding with Losartan. But not all of them are responsible for the toxic effect. Some give the desired effect of losartan but all are not associated with the beneficial effect. We had considered some of that protein for molecular docking. Firstly, these proteins had been searched and a 3D Structure model of human of those proteins had been downloaded from PDB. The crystal structure resolution was 2-2.5 Å. The raw proteins are not ready for use in docking. The protein downloaded had been complexed with another ligand, amino acid, fatty acid, synthetic molecule, water molecule or other non-functional protein. The desired portion was achieved by editing the protein molecule by FLARE software. Raw protein also contains an organic molecule attached with it, unnecessary spaces, a non-functional amino acid. It should be cleaned for further processing. It had been performed by PyMOL.The next step is energy minimization. Energy minimization has a direct impact on the overall efficiency of the docking protocol. All biomolecule and ligand can't be docked without energy minimization. It is performed to reduce the overall potential energy of the protein as well as the ligand. Docking is predicting an interaction. If the protein and the ligand are not stable, the interaction won't happen. The biological system (protein, ligand) is usually dynamic/not stable. The raw protein and ligand might have unfavourable bond length, bond angle or torsion angle. Unfavourable non-bonded interaction also may be present. Energy minimization had been conducted by Swiss PDB software. The last step performed was docking. The prepared final protein and ligand are docked in PyRX. There are many proteins predicted in Swiss Target Prediction. But all of them couldn't be docked for some technical difficulties. The proteins were too large for energy minimization and could not give a favourable conformation for docking. That's why the most significant proteins responsible for desired and undesired effects are docked. The proteins used in docking are Endothelin receptor, Tyrosine-protein kinase ABL and PPAR-gamma. Discovery studio was used to visualize the docked molecule. The same procedures were followed for Hydrochlorothiazide, but it exerts no toxicity profile on Protox. So, Hydrochlorothiazide is considered safe and hence docking was not performed for this drug.

Result

The objective of the research is to predict the toxicity and efficacy profiling of Losartan potassium and Hydrochlorothiazide combination drug. We utilize the docking approach to attain our goal. We had been considered the drugs separately for docking and recorded the binding affinity, binding site, binding strength, RMSD and other relevant factors.

Interaction of ligands with their binding sites can be characterized in terms of Binding affinity and is used to determine which ligand has a stable complex with protein. More negative value or lower binding affinity represents a more stable ligand-receptor interaction. In general, high-affinity ligand binding results from the greater intermolecular force between ligand and its receptor while low-affinity results from the less intermolecular force between ligand and it's the binding site. If the binding energy is a negative value it means the ligand was bound spontaneously with the protein and if it gives positive value it represents that binding is energy consuming and it occurs if the required energy is available.

Root Mean Square Deviation (RMSD) value is used to validate the docking protocol. It is an important factor for analyzing the stability of protein and predicting conformational changes of the protein. RMSD value depends on the binding interaction and energy between ligand and protein. An optimized protein should have the lowest RMSD value (ideally less than 1.5 or 1 Angstrom). Lower the RMSD value higher the accuracy of docking.

Toxicity Profiling of Losartan

Peroxisome Proliferator-activated Receptor Gamma (PPAR – gamma)

Table 1.Docking of Losartan with PPAR-gamma

Docking Algorithm	Autodockvina
Docking software	PyRx (version 0.8)
Docking method	Blind (maximum search space)
Protein	PPAR-gamma
Protein preparation	Flare, Pymol
Observation	Discovery studio Visualizer 2016

Table 2.Docking of Losartan with PPAR-gamma

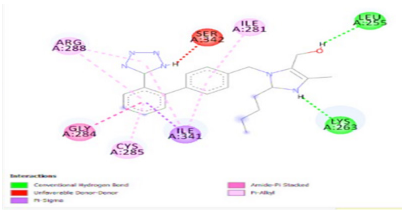
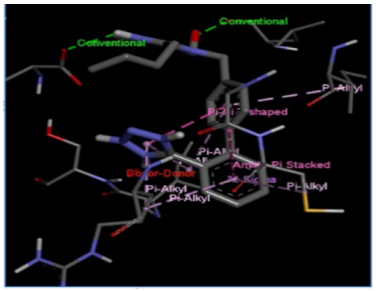
Vina Binding Affinity	-7.6 kcal/mol
RMSD/ub	0
RMSD/lb	0
2D structure	
3D structure	

Table 3.Docking of Losartan with PPAR-gamma

S. No.	Type of amino acid	PDB name	Full name	Hydrophobicity	PKa	Avg. isotopic displacement
1.	Arginine	ARG	A:288	-4.5	12	55.677
2.	Serine	SER	A:342	-0.8		48.004
3.	Isoleucine	ILE	A:281	4.5		41.606
4.	Cysteine	CYS	A:285	2.5	9	51.356
5.	Leucine	LEU	A:255	3.8		45.033
6.	Lysine	LYS	A:263	-3.9	10.4	85/734
7.	Isoleucine	ILE	A:341	4.5		34.601
8.	Glycine	GLY	A:284	-0.4		48.634

Tyrosine Kinase ABL

Table 4.Docking of Losartan with Tyrosine Kinase ABL

Docking Algorithm	Autodockvina
Docking Software	PyRx (version 0.8)
Docking Method	Blind (maximum search space)
Protein	Tyrosine kinase ABL
Protein Preparation	Flare, Pymol
Observation	Discovery studio Visualizer 2016

Hydrochlorothiazide Toxicity

Hydrochlorothiazide is considered a safe drug. Protox had been used for toxicity prediction of a particular molecule. We had been used Protox and Swiss Target Prediction for both drug molecule. But Protox did not give any toxicity profile prediction for Hydrochlorothiazide. It had shown no toxicity. Swiss Target Prediction algorithm gives a list of protein with which drug can interact. So, from the list of protein of both Losartan and Hydrochlorothiazide, there was no common protein found to interact. So, Hydrochlorothiazide had been dismissed from the docking procedure.

Table 5.Docking of Losartan with Tyrosine Kinase ABL

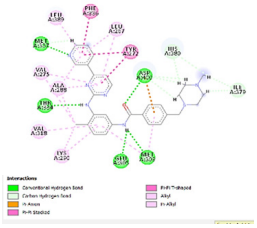
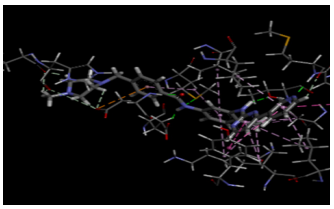
Vina Binding Affinity	-6.9 kcal/mol
rmsd/ub	0
rmsd/lb	0
2D structure	
3D structure	

Table 6.Docking of Losartan with Tyrosine Kinase ABL

S. No.	Type of amino acid	PDB name	Full name	Hydrophobicity	PKa	Avg. Isotopic Displacement
1.	Phenylalanine	PHE	A:336	2.8		32.575
2.	Tyrosine	TYR	A:272	-1.3	10	42.871
3.	Glycine	GLY	A:284			
4.	Aspartic acid	ASP	A:400	-3.5	3.9	41.37
5.	Histidine	HIS	A:380	-3.2	6	41.446
6.	Methionine	MET	A:309	1.9		41.202
7.	Isoleucine	ILE	A:379	4.5		45.124
8.	Glutamic acid	GLU	A:305	-3.5	4.3	43.13
9.	Methionine	MET	A:337	1.9		32.67
10.	Valine	VAL	A:318	4.2		37.944
11.	Threonine	THR	A:334	-0.7		41.523
12.	Alanine	ALA	A:288	1.8		36.28
13.	Leucine	LEU	A: 389	3.8		30.573
14.	Valine	VAL	A: 275	4.2		39.297

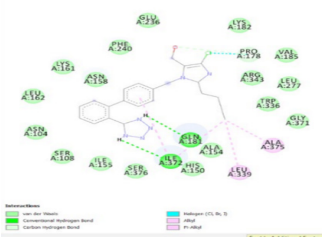
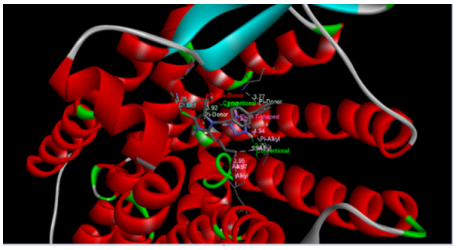
Efficacy Profiling of Losartan Potassium

Losartan is typically an anti-hypertensive drug. It shows its desired action by interacting with lots of protein molecule. From those, we had been chosen one suitable protein for docking, as all molecules do not fit the docking approach. Endothelin receptor is one of the fitting molecules for docking. Here is a representation of docking of Losartan and Endothelin receptor:

Table 7.Docking of Losartan with Endothelin receptor

Docking Algorithm	Autodockvina
Docking Software	PyRx (version 0.8)
Docking Method	Blind (maximum search space)
Protein	Endothelin receptor
Protein Preparation	Pymol
Observation	Discovery Studio Visualizer 2016

Table 8.Docking of Losartan with Endothelin receptor

Vina Binding Affiniy	-7.2 kcal/mol
RMSD/ub	0
RMSD/lb	0
2D Structure	
3D structure	

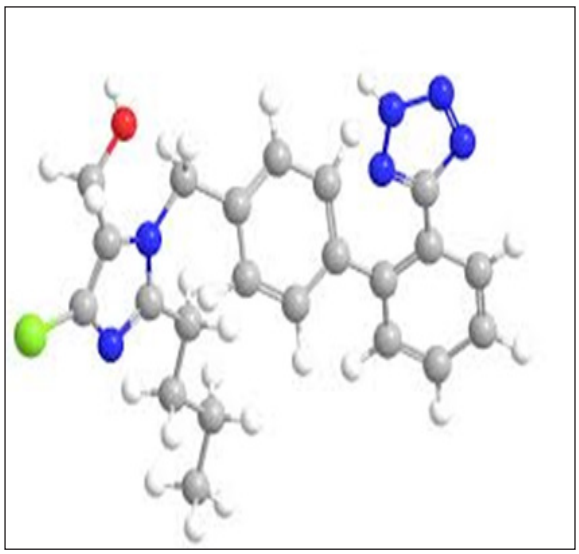


Figure 1.3D structure of Losartan

Figure 2.3D structure of Hydrochlorothiazide

Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	immuno	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Active	1.0
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Active	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Active	1.0
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.88
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.70
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.96
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99

Figure 3.Basic idea about toxicity of Losartan Potassium in Protox

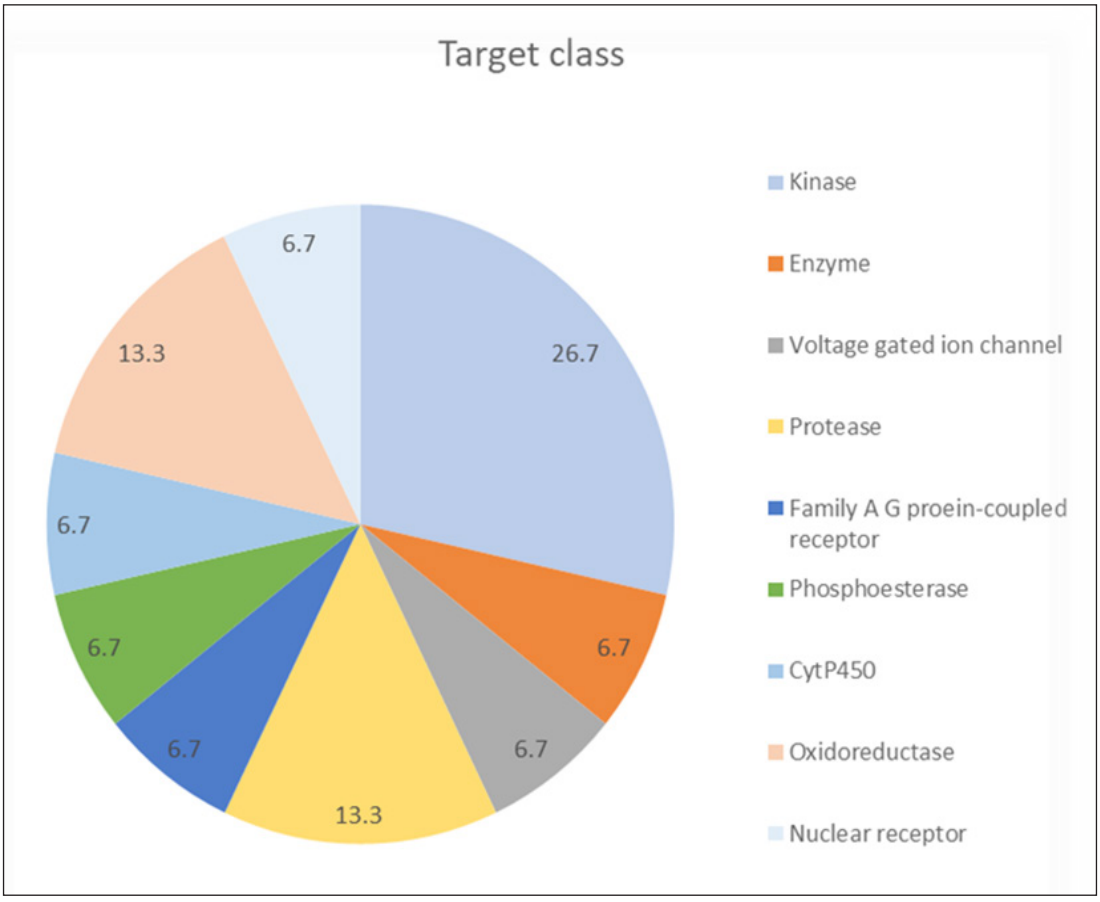


Figure 4.A Pie chart indicating the repartition of Target Class of protein of Losratan

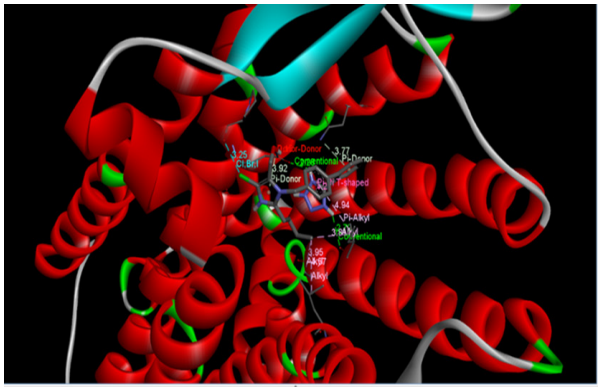


Figure 5.Docking of Endothelin receptor with Losartan

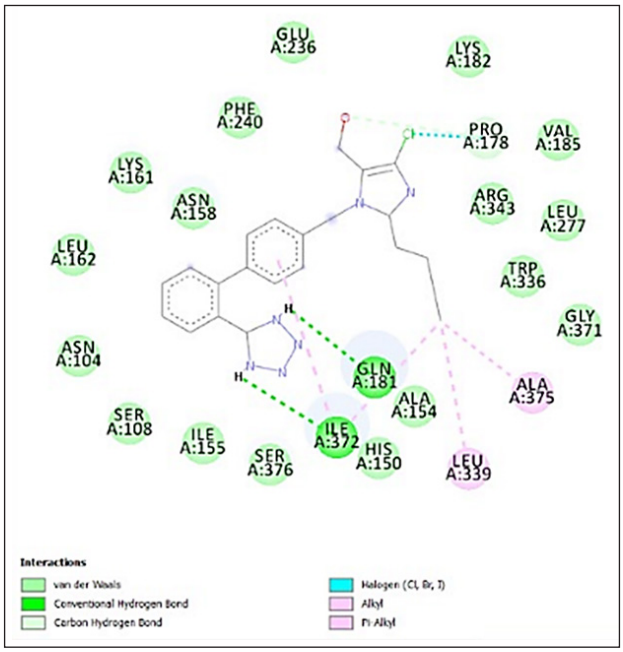


Figure 6.Docking of Endothelin receptor with Losartan

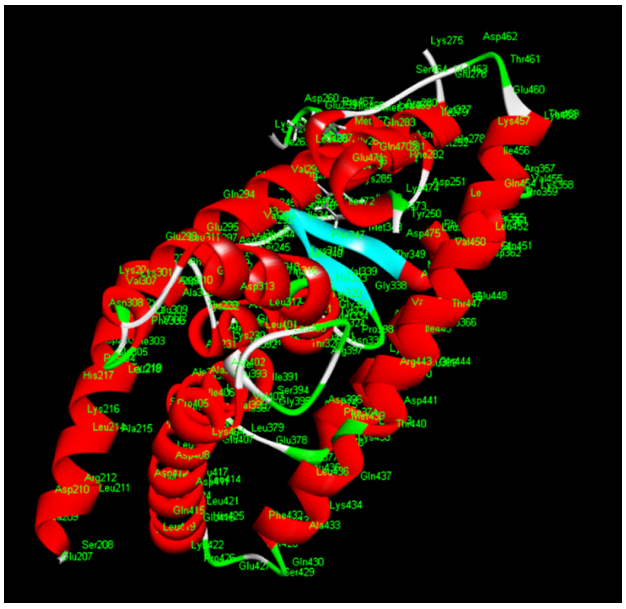


Figure 7.Docking of Losartan with PPAR-gamma

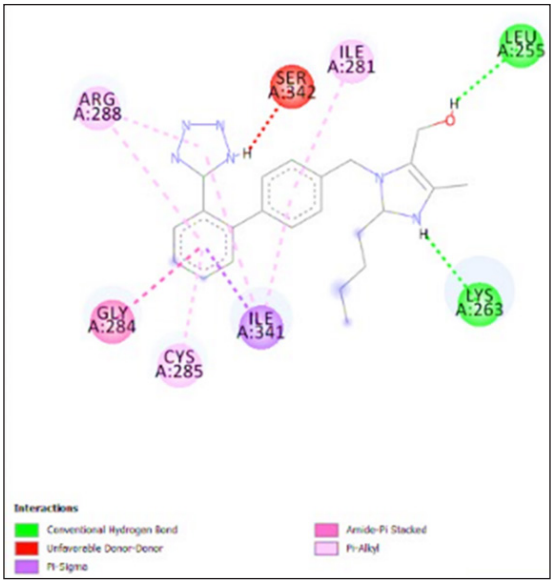


Figure 8.Docking of Losartan with PPAR-gamma

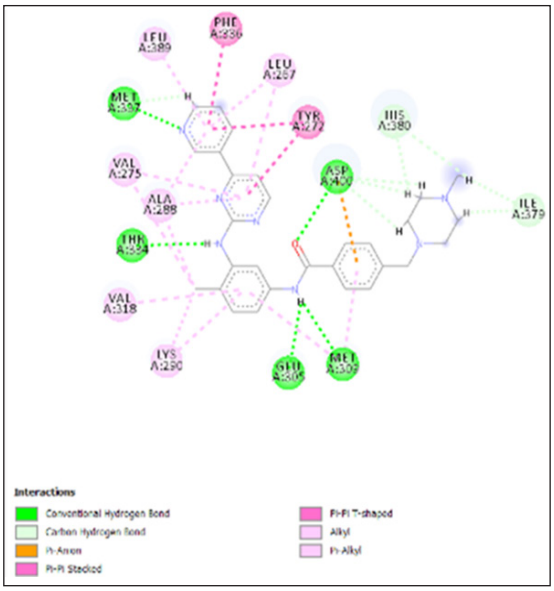


Figure 9.Docking of Losartan with Tyrosine kinase ABL

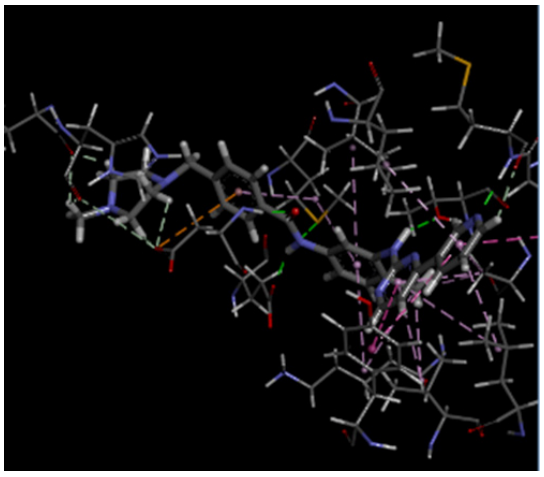


Figure 10.Docking of Losartan with Tyrosine kinase ABL

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.92
Toxicity end points	Carcinogenicity	carcino	Inactive	0.86
Toxicity end points	Immunotoxicity	immuno	Inactive	0.94
Toxicity end points	Mutagenicity	mutagen	Inactive	0.96
Toxicity end points	Cytotoxicity	cyto	Inactive	0.69
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.99
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.99
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0

Figure 11.Basic idea about toxicity of Hydrocholorothiazide in Protox

Discussion

Docking is a computational determination of binding affinity between molecules (usually protein and ligand). Binding affinity gives an idea of a stable complex. The interaction of the ligand with their binding site can be characterized by binding affinity. The more negative or lower the affinity the more stable the ligand-protein complex we get. Losartan is a widely used antihypertensive drug. It gives its desired effect by interacting with many proteins. Endothelin receptor is one of the suitable receptors for docking. Losartan binds strongly with the endothelin receptor, as it gives a good binding affinity in AutoDock Vina. But from Protox it had been seen that Losartan is accountable for immunotoxicity. Proteins named Peroxisome Proliferator activated receptor (PPAR) gamma and Tyrosine Kinase ABL indebted to immunotoxicity had been docked. From AutoDock Vina binding affinity we had been found that PPAR gamma forms a more stable complex with losartan than others. PPAR gamma possesses more negative binding affinity than Tyrosine Kinase ABL as well as endothelin receptor. All of these proteins creates Conventional Hydrogen Bond, van der Waals interaction, Pi sigma, Pi alkyl, unfavourable donor-donor interaction with Losartan molecule. These bond can cause reversible or irreversible distortion of the protein molecule and can cause toxicity. Hydrochlorothiazide is deliberated as a safe drug since it possesses no toxicity in Protox and Swiss Target Prediction. There was no common protein found for both drugs. So, it can be stated that Hydrocholorothaizide gives its desired action without causing any unwanted effect and doesn't interfere with Losartan's efficacy or toxicity.

Losartan Potassium and Hydrochlorothiazide combination therapy possesses its desired therapeutic action by acting separately. They both have a different mechanism of action. Toxicity governed by this combination drug is quite serious and is mainly acquainted by Losartan. So, this combination therapy should be monitored thoroughly and more research is required to overcome the problems.

Conclusion

Hypertension is one of the most alarming diseases now. About 1.13 billion people worldwide have hypertension. Most of the people remain undiagnosed at a preliminary stage. Uncontrolled hypertension increases the risk of heart attack, stroke and premature death. Hypertension is

called a 'silent killer'. So it should be well handed. Losartan potassium is used to treat hypertension and protect against kidney damage due to diabetes. Losartan is the second prescribed generic in anti-hypertensive drugs in terms of unit. Moreover, it is an INN drug. It is approved by the US in 1995. Losartan is taken for a long time. So any side effect can cause serious injury to health. From our, in silico research, it can be stated that Losartan can cause immunotoxicity. Though it is predicted it should be under research so that all limitations can be overcome. Hydrochlorothiazide is used in combination with other drugs to increase its efficacy. Alone Hydrochlorothiazide does not cause any toxicity but toxicity possessed by Losartan can't be minimized by Hydrochlorothiazide. So, the total toxicity we get from the combination therapy is mainly due to Losartan. So, more studies should be carried out to get sufficient knowledge and make the drug safer.

Acknowledgement

We would like to thank Department of Pharmacy of Jahangirnagar University to uphold their help and support throughout our research project.

Conflict of Interest: None

References

1. Yu W, MacKerell AD. Computer-Aided Drug Design Methods. *Methods in molecular biology (Clifton, NJ)*. 2017; 1520: 85-106.
2. Surabhi S, Singh BK. Computer Aided Drug Design: An Overview. *Journal of Drug Delivery and Therapeutics* 2018; 8: 504-509.
3. Toh R, Ishida T, Nishimura K et al. Comparison of medium-dose losartan/ hydrochlorothiazide and maximal-dose angiotensin II receptor blockers in the treatment of Japanese patients with uncontrolled hypertension: the Kobe-CONNECT Study. Hypertension research. *Official Journal of the Japanese Society of Hypertension* 2012; 35(11): 1080-1086.
4. Yuan S, Chan HCS, Hu Z. Using PyMOL as a platform for computational drug design. *WIREs Computational Molecular Science* 2017; 7(2): e1298.
5. Sica DA, Gehr TW, Ghosh S. Clinical pharmacokinetics of losartan. *Clinical pharmacokinetics* 2005; 44(8): 797-814.
6. Goa KL, Wagstaff AJ. Losartan potassium: a review of its pharmacology, clinical efficacy and tolerability in the management of hypertension. *Drugs* 1996; 51(5): 820-845.
7. Kim KS, Fan WH, Kim YD et al. Effectiveness of open-label losartan/hydrochlorothiazide combination therapy in Asian patients with hypertension not controlled with ACE inhibitor or ARB monotherapy. Hypertension research. *Official Journal of the Japanese Society of Hypertension* 2009; 32(6): 520-526.
8. Watanabe LA, Wei M, Sun N et al. Effect on blood pressure control of switching from valsartan monotherapy to losartan/ hydrochlorothiazide in Asian patients with hypertension: results of a multicentre open-label trial. *Current medical research and opinion* 2006; 22(10): 1955-1964.
9. Meno H, Inou T, Tanaka M et al. Antihypertensive efficacy of the losartan/ hydrochlorothiazide combination and its effect on plasma B-type natriuretic peptide in hypertensive patients uncontrolled by angiotensin II type 1 receptor antagonist-based therapy: a multicentre prospective observational study. *Clinical Drug Investigation* 2012; 32(3): 171-8.
10. Rakugi H, Tsuchihashi T, Shimada K et al. Efficacy and safety of fixed-dose losartan/ hydrochlorothiazide/ amlodipine combination versus losartan/hydrochlorothiazide combination in Japanese patients with essential hypertension. *Clinical and experimental hypertension* (New York, NY: 1993). 2015; 37(3): 260-206.
11. Burrell LM. A risk-benefit assessment of losartan potassium in the treatment of hypertension. *Drug Safety* 1997; 16(1): 56-65.
12. Goldberg A, Sweet C. Efficacy and safety of losartan. *The Canadian journal of cardiology* 1995; 11 Suppl F:27f-32f.
13. Manolis AJ, Grossman E, Jelakovic B et al. Effects of losartan and candesartan monotherapy and losartan/hydrochlorothiazide combination therapy in patients with mild to moderate hypertension. *Clinical Therapeutics* 2000; 22(10): 1186-1203.
14. Kohvakka A, Salo H, Gordin A et al. Antihypertensive and Biochemical Effects of Different Doses of Hydrochlorothiazide Alone or in Combination with Triamterene. *Acta Medica Scandinavica* 1986; 219(4): 381-386.
15. Soffer BA, Wright JT, Pratt JH et al. Effects of Losartan on a Background of Hydrochlorothiazide in Patients With Hypertension. *Hypertension* 1995; 26(1): 112-117.
16. Daina A, Michielin O, Zoete V. Swiss Target Prediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research* 2019; 47(W1): W357-W64.
17. Ruilope LM, Simpson RL, Toh J, Arcuri KE, Goldberg AI, Sweet CS. Controlled Trial of Losartan Given Concomitantly with Different Doses of Hydrochlorothiazide in Hypertensive Patients. *Blood Pressure* 1996; 5(1): 32-40.
18. Berglund G, Andersson O. Low doses of hydrochlorothiazide in hypertension. *European Journal of Clinical Pharmacology* 1976; 10(3): 177-182.
19. Neutel JM, Littlejohn TW, Chrysant SG et al. On

behalf of the Telmisartan Study G. Telmisartan/ Hydrochlorothiazide in Comparison with Losartan/ Hydrochlorothiazide in Managing Patients with Mild-to-Moderate Hypertension. *Hypertension Research* 2005; 28(7): 555-563.

20. Morris GM, Lim-Wilby M. Molecular docking. *Methods in molecular biology* (Clifton, NJ) 2008; 443: 365-382.
21. Schoenberger JA. Losartan with hydrochlorothiazide in the treatment of hypertension. *Journal of hypertension Supplement: Official Journal of the International Society of Hypertension* 1995; 13(1): S43-S47.
22. Wang YL, Xiao JW, Suzek TO et al. PubChem: A public information system for analyzing bioactivities of small molecules. *Nucleic Acids Research* 2009; 37: W623-W633. doi: 10.1093/nar/gkp456.